Sex Differences in Fear Conditioning and Extinction

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I declare that this thesis is my own work and that, to the best of my knowledge and belief, it does not contain material from published sources without proper acknowledgement, nor does it contain material which has been accepted for the award of any other higher degree or graduate diploma in any university.

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Hollie Blackley

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Abstract

Females have a known vulnerability to developing anxiety disorders. Greater fear conditioning and impaired fear extinction are proposed underlying mechanisms of anxiety disorder development. The aim of the current study was to examine sex differences in fear conditioning, extinction and reinstatement of fear whilst controlling for levels of sex hormones. It was anticipated that females, tested when their estrogen and progesterone is low, would have reduced fear conditioning but impaired fear extinction, compared to males. Skin conductance and ratings of threat expectancy were recorded for 36 undergraduates who underwent a differential fear conditioning, extinction and reinstatement paradigm. Results suggest there were no sex differences in the overall magnitude of fear acquisition and extinction for SCR amplitude. However, males displayed greater generalised SCR amplitude at the start of extinction, and more rapid fear SCR reduction in this phase, though not only to the feared stimulus, and greater reinstatement of fear for SCR amplitude. On the other hand, females displayed greater threat expectancy ratings in the acquisition, extinction, and reinstatement phases. These findings are broadly consistent with previous literature and highlight an interesting differential response between SCR and cognitive threat expectancy between males and females during fear conditioning, extinction and reinstatement.

Anxiety disorders are one of the most common mental disorders; affecting one in five adults in Australia and the United States (Kessler et al., 2005; McEvoy, Grove, & Slade, 2011). Therefore, anxiety disorders represent a significant burden of disease. Epidemiological studies reveal that females are twice as likely as males to develop an anxiety disorder (McLean, Asaani, Litz, & Hofmann, 2011; Parker & Brotchie, 2004). Therefore, exploring the underlying mechanisms of female vulnerability to anxiety disorders is warranted. Convergent theoretical models propose that core mechanisms underlying the development of anxiety disorders are greater fear conditioning and impaired fear extinction (Graham & Milad, 2011). Recently, there has been an increasing emphasis on examining sex differences in fear conditioning and extinction, but findings are often inconsistent (Lebron-Milad & Milad, 2012). This may be due to a failure to control for sex hormones, such as estrogen, which may impact on fear conditioning and extinction. Therefore, this study aims to examine sex differences in fear conditioning and extinction using a fear conditioning and extinction paradigm whilst controlling for sex hormones.

Differential Fear Conditioning and Extinction

In a laboratory setting, fear conditioning and extinction has frequently been examined using a differential fear conditioning and extinction paradigm (Gazendam & Kindt, 2012; Milad et al., 2006; Milad, Orr, Pitman, & Rauch, 2005). In this paradigm, the to-be conditioned stimulus (CS+) is repeatedly paired with an aversive stimulus (unconditioned stimulus; US; e.g. electric shock) that elicits a fearful response known as the unconditioned response (UR). Fearful responses include freezing in rodents, and a skin conductance response (SCR) or startle response in humans. The learned association between the CS+ and the US is such that, following the acquisition phase, the CS+ alone will induce a fearful response, which is known

as the conditioned response (CR). In the extinction phase, the CS+ is presented alone a number of times, with no US, which diminishes the association and therefore extinguishes the CR which is reflected in reduced SCR amplitude. In this paradigm, a second stimulus, known as the CS-, is never paired with the US and so the differential response to the CS+ and CS- reflects conditioning or extinction of fear (see Figure 1). According to Graham and Milad (2011), there is much cross-species evidence that supports this paradigm, that is, this model is consistent across both non-human animal and human studies.

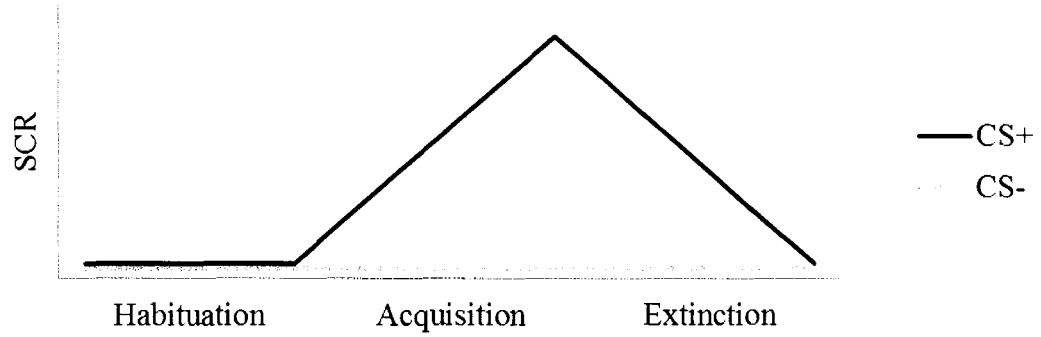


Figure 1. Expected SCR in the Habituation, Acquisition and Extinction Phases of a Fear Conditioning and Extinction Paradigm.

In a real world setting, a fear conditioning model of an anxiety disorder, such as Post Traumatic Stress Disorder (PTSD), would suggest that previously neutral objects that are present at the scene of a traumatic event become associated with the feared response, such that following the trauma these objects alone will induce a fearful response (Inslicht et al., 2013; Pitman et al., 2012; Shin & Liberzon, 2010). Although this model fits well with the mechanisms involved in the development of PTSD, the model can also be helpful in examining the underlying mechanisms of

other anxiety disorders as it facilitates the development of an anxiety reaction in a laboratory setting (Graham & Milad, 2011).

Additionally, developing a fear of a threatening situation or object is functional and adaptive (Zorawski, Cook, Kuhn, & LaBar, 2005). However, when an individual fails to learn that a situation or object no longer indicates threat, the fear is no longer functional or adaptive. Therefore, although fear conditioning contributes, it is not a sufficient explanation for the development of anxiety disorders. For instance, not every individual who experiences trauma or faces threat develops PTSD. Therefore, failure to extinguish conditioned fear is also thought to play a significant role in the development and maintenance of anxiety disorders (Hofmann, 2008; Rothbaum & Davis, 2003).

Differential fear extinction is also thought to mirror the mechanisms that underlie exposure therapy (Graham & Milad, 2011; Rothbaum & Davis, 2003). This is because confronting a feared stimulus repeatedly without an aversive consequence is equivalent to confronting a CS without a US, as both lead to reduction of the fear associated with the stimulus (Felmingham et al., 2007). The efficacy of exposure therapy has been demonstrated in randomized controlled trials and meta-analyses (Hofmann & Smits, 2008) and as such, exposure therapy is considered a first-line treatment of anxiety disorders.

Therefore, understanding fear conditioning and extinction may provide greater insight into mechanisms underlying anxiety disorders and their treatment with exposure therapy (Graham & Milad, 2011). Furthermore, identifying sex differences may enhance our understanding of the greater prevalence of anxiety disorders in women, compared to men and if they have fundamental differences in fear conditioning or extinction, this may reflect a vulnerability or risk factor for an

anxiety disorder.

Sex Differences in Fear Conditioning and Extinction

According to Lebron-Milad and Milad (2012), despite the known female vulnerability to anxiety disorders, the vast majority of studies examining the underlying mechanisms of anxiety disorders have used only male subjects (e.g., Anglada-Figueroa & Quirk, 2005; Ciocchi et al., 2010; Herry & Garcia, 2002; Laurent, Marchand, & Westbrook, 2008; Milad & Quirk, 2002; Morgan & LeDoux, 1995, 1999; Quirk, 2002; Sierra-Mercado, Corcoran, Lebron-Milad, & Quirk, 2006). Sex differences have become a focus of more recent animal and human research. However, studies to date have found little convergent evidence. For instance, the results of research involving animal subjects suggest that males have greater fear conditioning than females (e.g., Aguilar et al., 2003; Maren, De Oca, & Fanselow, 1994; Wiltgen, Sanders, Behne, & Fanselow, 2001). However, Dalla, Papachristos, Whetstone, and Shors (2008) found that female rats displayed faster eye-blink conditioning than male rats. Notably, markedly less research has examined sex differences in extinction learning (e.g., Baran, Armstrong, Niren, & Conrad, 2010). However, in a study involving both male and female rats, Baran, Armstrong, Niren, Hanna, and Conrad (2009) found that chronic stress impaired extinction recall (i.e., the recall of extinction learning) in male rats, comparative to female rats.

The relatively few studies that have examined sex differences in human participants have also yielded non-convergent evidence. Early studies, such as Guimaraes, Hellewell, Hensman, Wang, & Deakin (1991) found females had higher SCRs than males, particularly during conditioning. While Zorawski et al., 2005 found no sex differences in conditioning or extinction. Additionally, Jackson, Payne, Nadel, and Jacobs (2006) found that exposure to a stressor prior to conditioning

facilitated fear conditioning in men, whereas for women, stress appeared to impede differential conditioning.

Given the differential rate of anxiety disorders between males and females and that sex differences have been found, albeit non-convergent, studies examining fear conditioning and extinction should, at the very least, consider sex differences (Dalla & Shors, 2009). Additionally, early studies did not examine or control for hormonal changes, and there is now increasing evidence that sex hormones, such as estrogen, impact fear conditioning and extinction (e.g., Milad et al., 2006; 2010). Therefore, more recent studies have considered sex hormones and the menstrual cycle.

The Impact of Estrogen and the Menstrual Cycle

Recent studies suggest a role of estrogen in influencing fear conditioning and extinction. The brain regions that have been associated with conditioning and extinction of fear, such as the amygdala and hippocampus, have been found to be sexually dimorphic (Goldstein et al., 2001). Additionally, these regions have been found to have high concentrations of estrogen receptors, indicating that estrogen levels likely impact on their function (Spencer et al., 2008; Walf & Frye, 2006).

As shown in Figure 2, the menstrual cycle is divided into two phases; the follicular phase which starts on the first day of menses and ends on the day of ovulation which, in a 28 day cycle, would be day 14; and the luteal phase which starts on the day after ovulation and ends on the day prior to the beginning of menses (Fehring, Schneider, & Raviele, 2006). As can be seen, the levels of estrogen and progesterone fluctuate across the menstrual cycle. Specifically, during the early follicular phase (days 1-6), both estrogen and progesterone are low and in the late follicular phase (days 7-14), estrogen has a peak while progesterone remains low, whereas, during the luteal phase (days 15 – 28) there is another peak in estrogen and

a marked increase in progesterone.

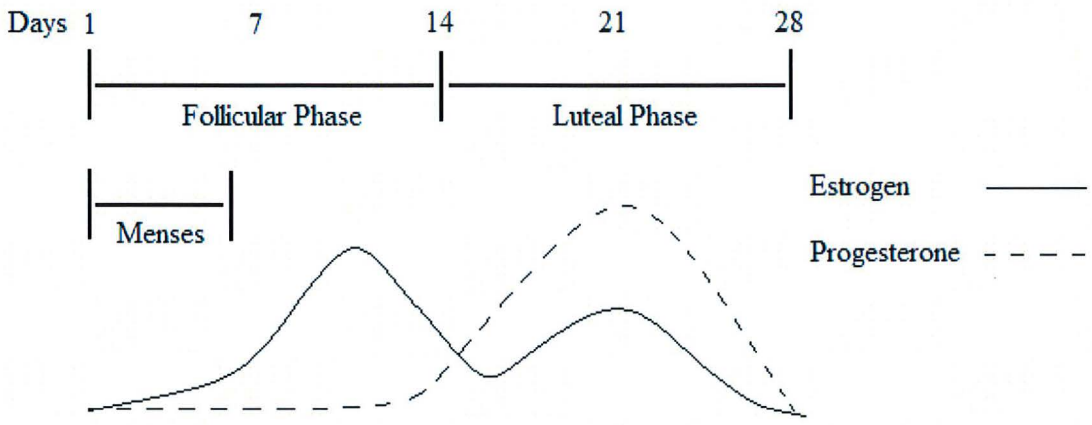


Figure 2. Estrogen and Progesterone Levels during the Follicular and Luteal Phases of the Menstrual Cycle.

Therefore, restricting the time of testing for females to menses ensures that they are within the early follicular phase (days 1-6) when both estrogen and progesterone levels are low. Additionally, the fluctuation of estrogen and progesterone throughout the menstrual cycle changes following menopause, which, according Henderson, Bernstein, Henderson, Kolonel, and Pike (2008) occurs between the ages of 40 and 58 years. Therefore, excluding individuals aged 40 years and above controls for these menopausal related hormonal changes.

Research that has controlled for or examined the effects of menstrual phase and levels of sex hormones, such as estrogen, on fear conditioning and extinction has predominantly been conducted in animals. For example, according to Shors, Beylin, Wood, and Gould (2000) female rats have higher rates of fear conditioning than do male rats, particularly when female rats have high estrogen levels, suggesting that estrogen enhances fear conditioning. Likewise, Leuner, Mendolia-Loffredo, and Shors (2004) found that high doses of estrogen given to ovarianectomised-female

rats enhanced eye-blink conditioning. Additionally, Jasnow, Schulkin, and Pfaff (2006) found that long-term estrogen treatment in female mice facilitated fear conditioning. In contrast, Gupta, Sen, Diepenhorst, Rudick, and Maren (2001) found that when ovarianectomised-female rats were administered estrogen replacement prior to conditioning, they froze significantly less in the first extinction trial than sham-injected-female rats, suggesting that high estrogen at the time of acquisition reduces fear conditioning. Furthermore, Chang et al. (2009) found no sex differences in conditioning but found that higher levels of estrogen enhanced fear extinction in female rats, comparative to male rats. Similarly, Milad, Igoe, Lebron-Milad, and Novales (2009) found that naturally-cycling-female rats that underwent extinction training when their estrogen and progesterone was high, had greater extinction recall than female rats who underwent extinction training when their estrogen and progesterone was low. Therefore, animal studies have shown inconsistent effects of estrogen on fear conditioning, though most animal studies suggest that high levels of estrogen facilitate fear extinction.

Preliminary human studies in this area suggest that the menstrual cycle impacts on fear conditioning (e.g., Inslicht et al., 2013; Milad et al., 2006; Vila & Beech, 1978) and extinction consolidation (e.g., Milad et al., 2006; 2010; Zeidan et al., 2011). However, the results of these studies have not been convergent. Specifically, Milad et al. (2006) found that males displayed greater conditioned responding than females in both the early- (low estrogen) and late-follicular phase (high estrogen) who did not differ. In relation to extinction learning, although Milad et al. (2006) did not find sex differences in early- or late-extinction on day one of their two-day paradigm, they did find that, on day two, late-follicular females (high estrogen) had poorer extinction recall than men and early-follicular women (low estrogen).

Whereas, in a subsequent human study, Milad et al. (2010) examined the effect of estrogen and progesterone by classifying female participants into high and low estrogen and high and low progesterone groups, using a median split. They found that females in the high estrogen group and men had better extinction recall than the low estrogen group. Similarly, Zeidan et al., (2011) found that females classified by median split as high estrogen had facilitated extinction recall comparative to females classified as low estrogen. However, as the latter two studies used serological data to conduct median split analyses, rather than using natural menstrual phases, further investigation is required into the impact of estrogen in a natural cycle. Therefore, given that most studies of sex difference in fear conditioning and extinction have not adequately controlled for sex hormones and recent evidence suggests that estrogen impacts on fear conditioning and extinction, further research is required to examine sex differences, whilst taking sex hormones into account (Milad & Quirk, 2012).

One known study also examined sex differences in recovery of fear (Milad, 2006) but found no differences, even when taking menstrual phase into account. However, this study examined recovery of fear due to changes in context, which is known as fear renewal, and therefore further research on sex differences in other mechanisms of fear recovery (e.g., reinstatement) has not been conducted.

Reinstatement of Fear

In early theories of extinction learning it was suggested that repeated exposure to the CS+ alone allows the individual to 'unlearn' the relationship between the CS and the US and this is known as the Simple Conditioning Model (Mowrer, 1939 as cited in Hofmann, 2008). However, more recently it has been proposed that repeated exposure to the CS+ alone leads to extinction learning whereby the previous conditioned response is inhibited due to the newly learned pairing between the CS

and the absence of an aversive consequence (Bouton, 2004). In fact, subsequent research involving rats has indicated that there are different neuronal circuits responsible for the acquisition of fear and the extinction or inhibition of fear (Herry et al., 2008). Additionally, the theory that fear extinction involves extinction learning is evidenced by the occurrence of relapse of fear as it demonstrates that the original learned relationship between the CS and US has not been 'unlearned'. For example, following exposure therapy, some clients with anxiety disorders have a return or relapse of anxiety symptoms (Brown & Barlow, 1995; Bruce et al., 2005; Boschen, Neumann, & Waters, 2009).

In a laboratory setting, relapse of fear involves a return of a fear of the CS+ following a successful fear extinction phase. According to Bouton (2002), there are four mechanisms of fear recovery or relapse; spontaneous recovery, reacquisition, renewal, and reinstatement of fear, though it is notable that these terms are often used interchangeably in the literature. For the purpose of the current study, the following definitions will be used. Spontaneous recovery of fear refers to the return of fear that occurs over time. Reacquisition refers to the return of fear that occurs when there is a subsequent pairing of the US and CS following extinction, for instance, a second acquisition phase following a conditioning and extinction paradigm or experiencing a second trauma in real life. Renewal refers to the return of fear that occurs when an acquired fear is extinguished in a different context and the individual is then exposed to the original conditioning context.

Finally, reinstatement, which will be the focus of the current study, refers to the return of fear that occurs when there is a subsequent occurrence of the US alone following extinction. For instance, an unprompted delivery of the US alone after the extinction phase in a laboratory setting or an individual re-experiences the fear

response after successful exposure therapy (e.g., Delamater, 1997; Dirikx, Hermans, Vansteenwegen, Baeyens, & Eelen, 2007; Rescorla & Heth, 1975). In a reinstatement paradigm, a second extinction phase is conducted after the reinstatement phase, which, for the purpose of this study, will be referred to as the re-extinction phase.

Notably, Sokol and Lovibond (2012) demonstrated that using a different US in the reinstatement phase still resulted in a return of fear, although they suggest that reinstatement may represent the appearance of a new fear, rather than the return of a previously held fear. However, Rachman and Whittal (1989) found that reinstatement did not occur when the stimuli used were too dissimilar (e.g., picture of spider and electrical stimulus) which Sokol and Lovibond (2012) suggest may indicate that a cognitive appraisal of the feared stimuli is involved in reinstatement.

Cognitive Variables

Cognitive variables are increasingly being recognised as impacting on fear conditioning, extinction and reinstatement. For example, according to Hofmann (2008), the underlying mechanism of extinction learning, and therefore of exposure therapy, is the opportunity for the individual to re-evaluate their level of threat expectancy. Indeed, in a study that examined the cognitive variable of threat expectancy, Lovibond, Davis, and O'Flaherty (2000) found that higher ratings of threat expectancy were associated with slower fear extinction. Similarly, Gazendam and Kindt (2012) found that inducing worry after fear conditioning also led to slower rates of fear extinction. According to Lovibond and Shanks (2002), extinction learning involves both low-level unconscious processing and higher-level conscious, cognitive processing. Therefore, cognitive variables such as threat expectancy are sometimes measured alongside skin conductance in fear conditioning and extinction paradigms.

Notably, sex differences have been found in cognitive variables. For instance, women have been shown to make greater assessments of threat than men (McLean & Anderson, 2009) and to have higher levels of catastrophic cognitions (e.g., worry, Nolen-Hoeksema, Larson, & Grayson, 1999), higher levels of rumination, and less perceived control over their emotions (Nolen-Hoeksema & Jackson, 2001). Additionally, in a study by Felmingham and Bryant (2012), men who received exposure therapy but did not receive concurrent cognitive therapy had greater relapse in PTSD symptoms after six months, comparative to women. Together, this provides evidence that cognitive factors may influence fear conditioning, extinction and reinstatement and that the effect of these cognitive variables may differ between males and females. Therefore, cognitive variables of interest such as threat expectancy, levels of catastrophic cognitions, and perceived ability to regulate emotion will also be included in the current study.

The Current Study

Given the previous inconsistences in evidence of sex differences in fear conditioning and extinction, the aim of this study was to examine sex differences in fear conditioning, extinction and reinstatement whilst controlling for sex hormone levels. Sex differences will be examined using both Skin Conductance and Threat Expectancy.

1. It is hypothesised that during the acquisition phase, males will be more reactive to the feared stimulus (CS+), reflected in higher levels of SCR amplitude and higher ratings of threat expectancy, compared to females.
2. It is hypothesised that females, tested in the early-follicular phase when estrogen is low, will display impaired fear extinction, reflected in higher levels of SCR amplitude and higher ratings of threat expectancy during the

early- and late-extinction sub-phases, compared to males.

3. This study will also examine whether there are sex differences in reinstatement of fear, reflected in differential SCR and threat expectancy ratings in the re-extinction phase, following the two un-cued presentations of the US. However, as no known studies have examined this previously a directional hypothesis will not be made.

Method

Participants

The sample comprised 36 (22 male, 14 female) undergraduate students aged between 18 and 39 years from the University of Tasmania who received course credit for their participation. Individuals who were taking medication, who had hypertension, a heart condition, epilepsy, a diagnosed psychological disorder, or who were currently pregnant, were excluded from the study. Participants were asked to abstain from consuming alcohol, using illicit drugs and excessive exercise in the 24 hours prior to participating. They were also asked to abstain from caffeine and smoking for 3 hours prior and eating for 1 hour prior to participating.

In order to control for the effect of estrogen and progesterone on the acquisition, extinction and reinstatement of fear, female participants were tested during the early follicular phase (defined as days 1-6 of a 28 day cycle, when menstruating), when both estrogen and progesterone are low (Goldstein et al., 2005). Additionally, as discussed earlier, all participants were under the age of 40 years in order to control for menopause-related hormone changes. Menstrual phase was confirmed by self-reported date of commencement of menses prior to and at the time of participation and, given budgetary constraints; serological hormone levels were not obtained.

Design

The study followed a 2 [Sex: Male, Female] x2 (CS type: CS+, CS-) x3 (Trial: 1, 2, 3, 4) mixed factorial design for Habituation and a 2 [Sex: Male, Female] x2 (CS type: CS+, CS-) x4 (Trial: 1, 2, 3, 4, 5) mixed factorial design for Acquisition, Early-Extinction, Late-Extinction and Re-Extinction. Separate analyses were conducted for the dependant variables of SCR and Threat Expectancy.

Materials

A computer running Inquisit 3.0.6.0 (Millisecond Software, 2011) was used to display the fear conditioning and extinction paradigm for participants to view and to collect the threat expectancy data. A separate computer running Lab Chart 7.3.7 (ADInstruments, 2012) was used to collect skin conductance data. The US was a 500 millisecond mild electrical stimulus administered using a Powerlab 16/35 Recording Bare Electrode (MLADDB30) attached to the first dorsal interosseous muscle of the dominant hand and generated by a PowerLab 16/35 Stimulus Isolator (FE180). Galvanised skin response (GSR) was measured using a PowerLab 16/35 GSR Amp (FE116) and GSR Finger Electrodes (MLT116F) placed on the intermediate phalange of the first and third fingers of the non-dominant hand.

As a measure of participants' current mood the Depression, Anxiety and Stress Scale (*DASS*; Lovibond & Lovibond, 1995, see Appendix A) was administered. This is a questionnaire consisting of 21 statements (e.g., I tended to over-react to situations, I felt I was close to panic) to which participants are required to rate on a 4-point Likert scale (1= Not at all, 4= Very much, or most of the time) how much each statement applied to them over the past week. This questionnaire yields three subscales; depression, anxiety and stress with Cronbach's α of .91, .84, and .90 for each subscale respectively.

To measure participants' catastrophic cognitions, the Catastrophic Cognitions Questionnaire - Modified (*CCQ-M*; Khawaja, Oei, & Baglioni, 1994; see Appendix A) was administered. This questionnaire consists of 21 occurrences (e.g., being ill, being angry, losing memory) that are sometimes believed to be dangerous and participants are required to rate on a five-point Likert scale (1= Not at all dangerous, 5= Extremely dangerous) how dangerous they believe each is to them (Cronbach's α > .83 for all subscales).

Emotion regulation was assessed using the Difficulties with Emotion Regulation Scale (*DERS*; Gratz, & Roemer, 2004; see Appendix A). This is a 36 item scale on which participants are required to rate on a five-point Likert scale (1= almost never, 5= almost always) how often emotion regulation related statements apply to them (e.g., When I'm upset, I feel like I am week; I pay attention to what I am feeling; Cronbach's α = .93).

Participants also completed a post-experiment questionnaire (see Appendix A) in which they rated the intensity of the electrical stimuli on a 5-point Likert scale (1= Not, 5= Very). They were also required to indicate how often each CS was followed by an electrical stimuli during the experiment (i.e., Never, Sometimes, Always).

Procedure

Ethics approval was obtained from the University of Tasmania's Human Research Ethics Committee (see Appendix B). After informed consent was obtained (see Appendix C for information and consent forms), participants completed the set of questionnaires including the DASS, the CCQ-M, and the DERS (see Appendix A). Participants then had small recording disks attached to their fingertips to measure SCR throughout the experiment and were instructed to keep their hands still on the desk during the study to reduce movement artefacts in skin conductance recordings.

Participants were then asked to select a level of the US that was "uncomfortable but not painful" to them. This was done by attaching the stimulator and delivering the lowest level of electrical stimulus (2mA), and subsequently increasing this level in small increments (0.5mA) until the participant reported that it felt uncomfortable but not painful.

Participants then watched a computer screen on which the differential fear conditioning and extinction paradigm was shown. The fear conditioning and extinction paradigm was an adaptation of that used by Milad et al. (2006). The CS+ and CS- were red and blue circles presented for 12 seconds, the selection which was randomly determined and counterbalanced across participants. In all phases, circles were presented in the middle of the white background of the 14-inch-computer screen to keep the context constant. A variable inter-trial interval was employed, ranging from 12-21 seconds (mean 16 seconds). Skin conductance was recorded for 2 seconds prior to the presentation of the CS, during the 12 second CS presentation and for 6 seconds following the US presentation. Additionally, throughout the study, participants used the mouse to rate their level of threat expectancy on an eleven-point Likert scale (-5 = certain no shock, 0 = uncertain, 5 = certain shock) displayed concurrently with each trial.

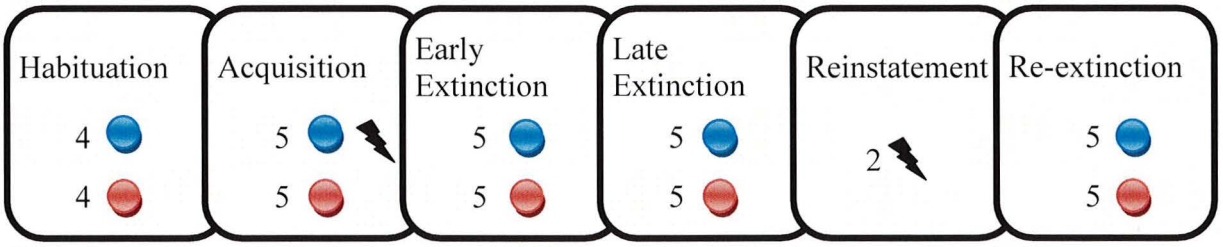


Figure 3. Trials in each of the phases of the differential conditioning, extinction, and reinstatement paradigm.

As reported in Milad et al. (2006) and shown in Figure 3, the habituation phase contained eight trials, four of each CS. The acquisition phase contained ten trials, five of each CS with the US occurring immediately following the offset of the CS+ (100% reinforcement). The extinction phase was divided into two sub-phases (Early- and Late-Extinction), which were separated by an approximate 1-minute rest period. These sub-phases each contained 10 trials, five of each CS and in this phase, the CS+ was not followed by the US on any trial. The reinstatement phase contained two US trials alone followed by a re-extinction phase which contained 10 trials, five of each CS, without any US presentations. All CS presentations were quasi-random, with no CS occurring more than twice in a row. Prior to the habituation phase, participants were advised that they would not receive any electrical stimuli. Prior to the acquisition, extinction and re-extinction phases, participants were told that they may or may not receive an electrical stimulus and during each trial, participants rated their level of threat expectancy. At the completion of the paradigm, participants completed the post-experiment questionnaire which aimed to ascertain that they had gained awareness of the CS+/ US contingency and were then fully debriefed about the aims of the study.

Analysis

Demographic and questionnaire data were analysed using independent-samples *t*-tests to examine group differences. SCR and threat expectancy data were analysed using separate 2 [Sex: Male, Female] x2 (CS type: CS+, CS-) x3 (Time: 1, 2, 3) mixed factorial ANOVAs for the Habituation phase and a 2 [Sex: Male, Female] x2 (CS type: CS+, CS-) x4 (Time: 1, 2, 3, 4) mixed factorial ANOVAs for the Acquisition, Early-Extinction, Late-Extinction and Re-Extinction phases. Notably, in order to directly compare results, Time 1, 2, 3, and 4 were obtained by averaging

SCR and threat expectancy ratings on trial 1 and 2, trial 2 and 3, trial 3 and 4, and 4 and 5, respectively, as was done by Milad et al. (2006). Sidak-adjusted pairwise comparisons were used to examine significant main effects and interactions, and green-house geisser corrections were used when sphericity was significant. Regression analysis was used to examine the relationship between the sex differences found in the SCR and threat expectancy data and the cognitive and demographic variables that females and males differed significantly on. Significance was set at $p <$ .05 and effect sizes (g and η_p^2) and 95% confidence intervals are also reported.

Results

Demographic and Clinical data

As shown in Table 1, males and females did not differ significantly with respect to age or depression as measured by the DASS. Additionally, there were no significant differences between males and females on subjective ratings of catastrophic cognitions, as measured by the *CCQ-M*, although groups differed at trend level with a moderate effect size. However, females rated themselves as significantly more anxious and stressed than males, as measured by the *DASS*, and as having significantly more difficulty regulating their emotions, as measured by the *DERS* (Gratz, & Roemer, 2004).

Skin Conductance

Data from participants (n = 2) who reported that they did not feel any electrical stimuli were excluded, as was data from participants (n = 2) whose skin conductance recording contained interference, leaving 32 participants (20 male, 12 female). The raw data was inspected and individual trials that were affected by artefacts were removed. On this basis, approximately 2% of trials were removed. Additionally, data was screened for outliers (defined as >3 standard deviations from the group mean),

and were replaced with a value just within three standard deviations of the group mean. On this basis, less than 1% of trials were replaced.

Table 1

Means, Standard Deviations and T-tests for males and females Age and Subjective Ratings on the DASS, CCQ-M and DERS.

	Males *M* *(SD)*	Females *M (SD)*	*t*	*df*	*p*	*95% CI* *Upper Bound*	*Lower Bound*	*g*
Age	24.6 yrs (5.6)	21.8 yrs (4.4)	1.57	34	.126	-0.82	6.34	0.54
DASS Depression	3.05 (3.4)	2.79 (1.9)	0.26	33	.794	-1.76	2.08	0.09
DASS Anxiety	1.67 (1.6)	4.71 (4.4)	-2.47	15.4	.026*	-5.68	-0.42	1.00
DASS Stress	3.48 (1.9)	5.50 (3.4)	-2.03	18.8	.056	-4.11	0.06	0.79
CCQ	54.38 (10.2)	61.76 (14.0)	1.80	33	.080	-15.76	0.95	0.62
DERS	73.76 (14.0)	85.43 (18.0)	2.16	33	.039*	-22.68	-0.65	0.74
$N = 36$								

Note: M = Mean, SD = Standard Deviation, CI = Confidence Interval, * = significant at the level of >.05.

Mean and peak Skin Conductance Level (SCL) amplitudes were obtained using a macro for each participant on each trial. To yield baseline SCL, the 2 seconds prior to all eight CS presentations in the Habituation phase was averaged for each participant. Skin Conductance Response (SCR) amplitude was then calculated for each trial by subtracting the mean SCL during the 2 seconds prior to onset of each CS from the highest SCL recorded during the 12 seconds of each CS presentation. Therefore, the SCR amplitude was the change from baseline SCL that occurs in

response to each CS presentation (e.g., Milad et al., 2005; 2006). This enabled the detection of the maximal increase in SCL that occurred for each CS. Additionally, unconditioned SCR was calculated by subtracting the mean SCL during the 12 second stimulus presentation from the highest SCL recorded during the 6 seconds following administration of the US. Each SCR was then square-root transformed to reduce heteroscedasticity, by using the absolute value and then returning the negative sign to negative SCR values. Following Milad et al. (2006) and given that trials were removed due to movement artefact, the data was smoothed using a running average between trials, that is, the average of trial 1 and 2, the average of trial 2 and 3, the average of trial 3 and 4, and the average of trial 4 and 5, which will be referred to as Time 1, Time 2, Time 3 and Time 4 respectively.

Shock Levels and Baseline Skin Conductance. As shown in Table 2, an independent-samples *t*-test showed no significant difference between males and females on the level of shock selected. Nor was there a significant difference between males and females on the subjective rating of shock intensity. Also, a one-way ANOVA showed no significant difference between males' and females' unconditioned SCR to the shocks in the acquisition phase. Therefore, any differences between groups in SCR across the phases of the paradigm cannot be attributed to shock level, perception of shock intensity, or unconditioned response to the shock. However, there was a trend toward an effect of Sex on baseline SCL whereby the males' baseline SCL was higher than the females. Therefore, baseline SCL will be considered in any sex differences detected.

Habituation. For skin conductance, a three-way mixed-factorial ANOVA showed a trend toward a main effect of Time, $F(1.28, 38.24) = 3.19$, $p = .072$, $\eta_{p2} = .096$, following a green-house geisser correction. Sidak-adjusted pairwise

comparisons revealed that although, overall, participants' SCR decreased between Time 1 ($M = .67$) and Time 2 ($M = .59$, $p = .187$, 95% CI [-0.03, 0.18]) and between Time 2 and Time 3 ($M = .53$, $p = .497$, 95% CI [-0.16, 0.05]), these differences did not reach significance.

Table 2

Means, Standard Deviations and T-tests for Males' and Females' Level of Shock Selected, Rating of Shock Intensity and Baseline Skin Conductance Level.

						95% CI		
	Males *M (SD)*	Females *M (SD)*	*t*	*df*	*p*	*Lower Bound*	*Upper Bound*	*g*
Level of shock	2.77 (0.82)	2.63 (0.83)	0.47	30	.645	-0.47	0.75	0.17
Rating of shock intensity	3.05 (1.05)	3.25 (0.62)	-0.68	29.9	.504	-0.80	0.40	0.22
Baseline SCL	2.68 (1.03)	1.83 (1.46)	1.93	30	.063	-0.05	1.75	0.71
	Males *M*	Females *M*	*F*	*df*	*p*			η_p^2
Unconditioned Response	1.66	1.63	0.02	1, 30	.889			.001

Note: M = Mean, SD = Standard Deviation, CI = Confidence Interval

The main effects of CS and Sex were non-significant (see Table D1 in Appendix D). Additionally, as shown in Figure 4, the three-way interaction between Sex, CS and Time was non-significant, $F(1.40, 42.05) = 0.13$, $p = .806$, $\eta_{p2} = .004$, following a green-house geisser correction. As were the interactions between Sex and CS, Sex and Time, and Time and CS (see Table D2 in Appendix D). Therefore, these interactions were not explored further.

Acquisition. For skin conductance, a three-way mixed-factorial ANOVA showed a significant main effect of CS, $F(1, 30) = 46.93$, $p < .001$, $\eta_p^2 = .610$,

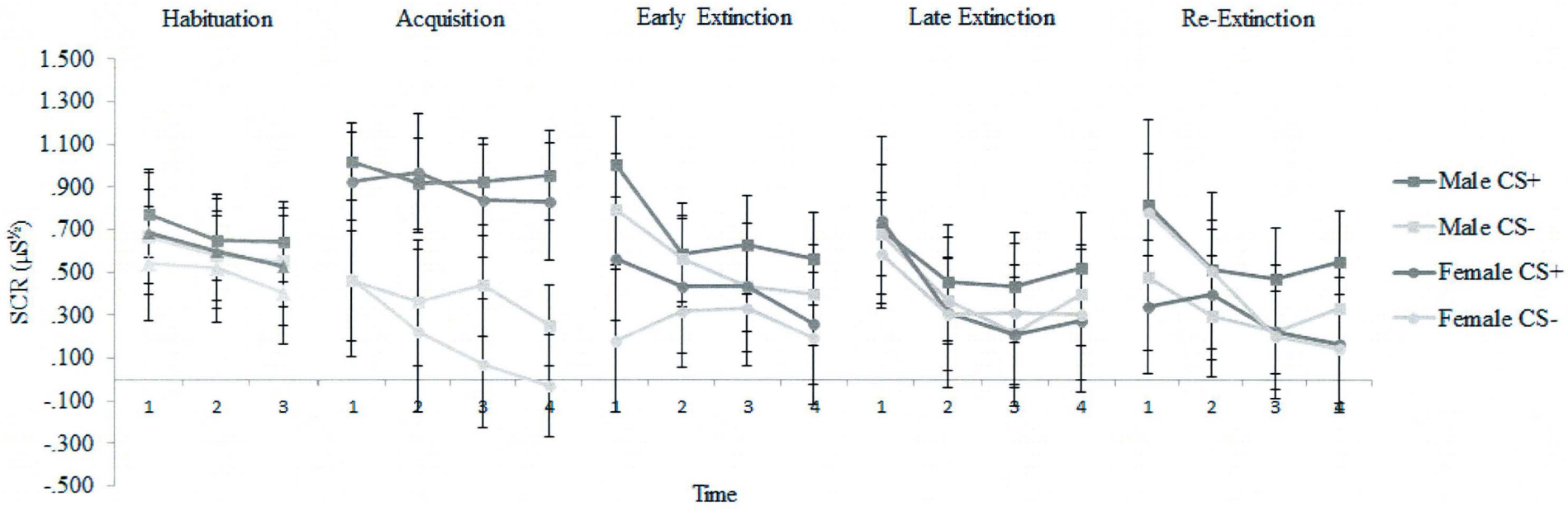
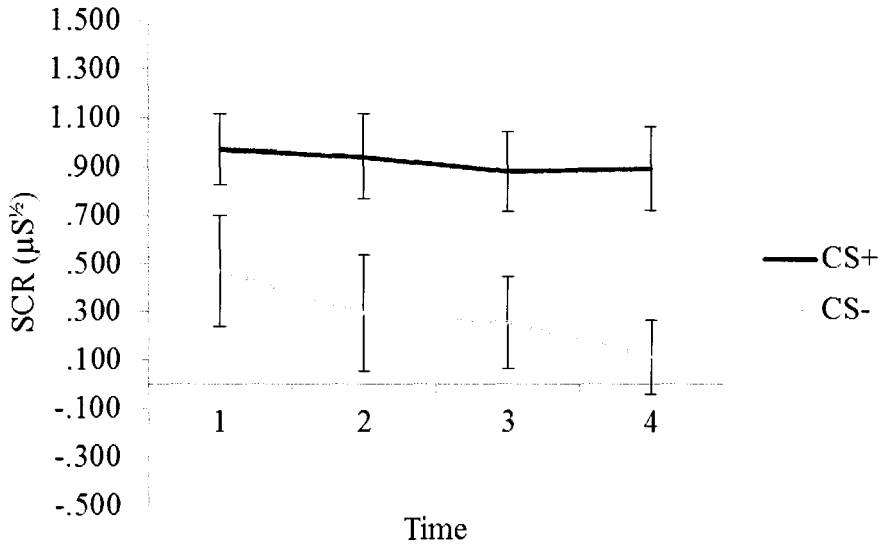


Figure 4. Skin Conductance Response (SCR) for Each CS at each Time point for Males and Females in the Habituation, Acquisition, Early-Extinction, Late-Extinction, and Re-Extinction Phases

indicating that overall participant's SCR to the CS+ (M = .92) was significantly higher than to the CS- (M = .28). There was also a main effect of Time, F(1.87, 56.18) = 4.37, p = .019, η_{p2} = .127, following a green-house geisser correction. However, sidak-adjusted pairwise comparisons revealed that, overall, participants' SCR did not change significantly between Time 1 (M = .72) and Time 2 (M = .62, p = .193, 95% CI [-0.03, 0.23]), between Time 2 and Time 3 (M = .57, p = .910, 95% CI [-0.09, 0.19]), or between and Time 3 and Time 4 (M = .50, p = .806, 95% CI [-0.22, 0.09]). Additionally, as shown in Figure 5, the interaction between Time and CS was non-significant, F(2.12, 63.53) = 1.37, p = .264, η_{p2} = .043, following a green-house geisser correction.



Note: Error bars are 95% confidence intervals.

Figure 5. SCR at each Time point for CS+ and CS- in the Acquisition Phase.

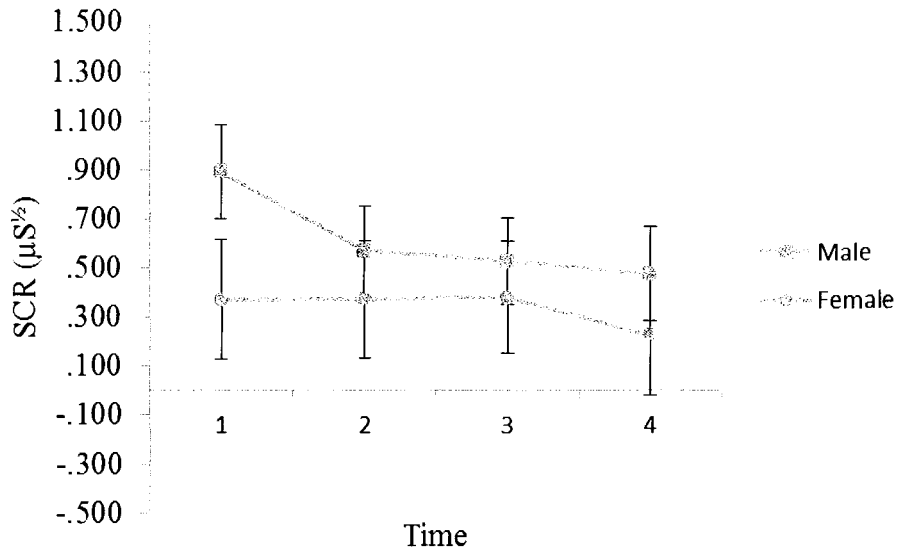
Additionally, the main effect of Sex was non-significant (see Table D3 in Appendix D). Also, as shown in Figure 4 (on page 22), the three-way interaction between Sex, CS and Time was non-significant, F(2.12, 63.53) = 0.70, p = .507, η_{p2}

= .023, following a green-house geisser correction. As were the interactions between Sex and CS, and Sex and Time (see Table D4 in Appendix D). Therefore, these interactions were not explored further.

Early-Extinction. For skin conductance, a three-way mixed factorial ANOVA revealed three significant main effects. First, a significant main effect of CS showed that overall, participant's SCR to the CS+ (M = .56) was significantly higher than to the CS- (M = .40), $F(1, 30) = 4.79$, $p = .037$, $\eta_{p2} = .138$. Second, a significant main effect of Sex showed that overall, the Male's SCR (M = .62) was significantly higher than the Female's SCR (M = .34), $F(1, 30) = 4.58$, $p = .041$, $\eta_{p2} = .132$. Third, a significant main effect of Time was found, $F(1.8, 54.59) = 7.98$, $p = .001$, $\eta_{p2} = .210$, following a green-house geisser correction.

However, as shown in Figure 6, the main effects of Time and Sex were subsumed by a significant interaction, $F(1.82, 54.59) = 4.16$, $p = .024$, $\eta_{p2} = .122$, following a green-house geisser correction. Sidak-adjusted pairwise comparisons broken down by Time revealed that males' SCR was significantly higher at Time 1 (M = .89) than at Time 2 (M = .57, $p < .001$, 95% CI [0.16, 0.49]), but were not significantly different between Time 2 and Time 3 (M = .53, p = .984, 95% CI [-0.13, 0.21]), or Time 3 and Time 4 (M = .48, p = .789, 95% CI [-0.17, 0.07]). In contrast, females' SCR was not significantly different between Time 1 (M = .37) and Time 2 (M = .37, p = .999, 95% CI [-0.22, 0.21]), or between Time 2 and Time 3 (M = .38, p = .999, 95% CI [0.23, 0.21]), but female's SCR at Time 3 was significantly higher than at Time 4 (M = .23, p = .045, 95% CI [-0.31, -0.01]). Notably, broken down by sex, pairwise comparisons revealed that males' mean SCR was significantly higher than females' at Time 1 (p = .002, 95% CI [0.21, 0.83]) but not at Time 2 (p = .197, 95% CI [-0.11, 0.50]), Time 3 (p = .309, 95% CI [-0.14, 0.44]), or Time 4 (p = .108,

95% CI [-0.06, 0.56]).



Note: Error bars are 95% confidence intervals.

Figure 6. SCR at each Time point for males and females in the Early-Extinction Sub-Phase.

Additionally, as shown in Figure 4 (on page 22), the three-way interaction between Sex, CS and Time was non-significant, $F(1.68, 50.49) = 0.62$, $p = .515$, $\eta_{p2} = .020$, following a green-house geisser correction. As were the interactions between Sex and CS and Time and CS (see Table D5 in Appendix D). Therefore, these interactions were not explored further.

Late-Extinction. For skin conductance, the mixed factorial ANOVA showed a significant main effect of Time, $F(1.99, 59.67) = 11.27$, $p < .001$, $\eta_{p2} = .273$, following a green-house geisser correction. Sidak-adjusted pairwise comparisons revealed that, overall, participants' SCR at Time 1 ($M = .67$) was significantly higher than at Time 2 ($M = .36$, $p < .001$, 95% CI [0.15, 0.48]), but were not significantly different between Time 2 and Time 3 ($M = .29$, $p = .870$, 95% CI [-0.11, 0.24]), or

Time 3 and Time 4 (M = .37, p = .554, 95% CI [-0.06, 0.23]).

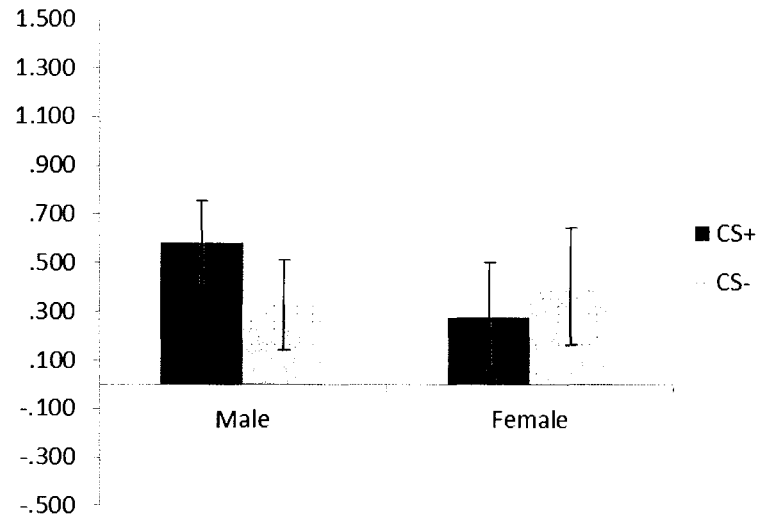
The main effects of CS and Sex were non-significant (see Table D6 in Appendix D). Additionally, as shown in Figure 4 (on page 22), the three-way interaction between Sex, CS and Time was non-significant, $F(1.85, 55.47) = 1.02$, p = .362, η_{p2} = .033, following a green-house geisser correction. As were the interactions between Sex and CS, Sex and Time, and Time and CS (see Table D7 in Appendix D). Therefore, these interactions were not explored further.

Re-Extinction. For skin conductance, a three-way mixed-factorial ANOVA showed a significant main effect of Time, $F(1.72, 51.81) = 5.92$, p = .007, η_{p2} = .165, following a green-house geisser correction. Sidak-adjusted pairwise comparisons revealed that participants' SCR at Time 1 (M = .60) trended toward being significantly higher than at Time 2 (M = .42, p = .073, 95% CI [-0.01, 0.36]), but was not significantly different between Time 2 and Time 3 (M = .28, p = .326, 95% CI [-0.07, 0.36]), or Time 3 and Time 4 (M = .29, p = .999, 95% CI [-0.14, 0.18]).

Additionally, the main effects of CS and Sex were non-significant (See Table D8 in Appendix D). However, as is shown in Figure 7, there was a significant CS by Sex interaction, $F(1, 30) = 7.14$, p = .012, η_{p2} = .192. Sidak-adjusted pairwise comparisons broken down by Sex revealed that the males' SCR to the CS+ (M = .58) was significantly higher than to the CS- (M = .33, p = .007, 95% CI [-0.43, -0.08]). However, the females' SCR to the CS+ (M = .28) was not significantly different from their SCR to the CS- (M = .40, p = .270, 95% CI [-0.10, 0.36]).

Additionally, as shown in Figure 4 (on page 22), the three-way interaction between Sex, CS and Time was non-significant, $F(2.07, 62.08) = 1.80$, p = .173, η_{p2} = .056, following a green-house geisser correction. As were the interactions between Sex and Time, and Time and CS (see Table D9 in Appendix D). Therefore, these

interactions were not explored further.



Note: Error bars are 95% confidence intervals.

Figure 7. SCR to the CS+ and CS- for males and females in the Re-Extinction Phase

Threat Expectancy

Habituation. For threat expectancy, a three-way mixed-factorial ANOVA showed a significant main effect of Time, $F(1.51, 45.18) = 3.69$, $p = .044$, $\eta_{p2} = .110$, following a green-house geisser correction. Sidak-adjusted pairwise comparisons revealed that, overall, participants' threat expectancy ratings were not significantly different between Time 1 ($M = .02$) and Time 2 ($M = -.17$, $p = .842$, 95% CI [-0.45, 0.82]), but ratings at Time 2 trended towards being significantly higher than at Time 3 ($M = -.72$, $p = .063$, 95% CI [-1.12, 0.02]).

The main effects of CS and Sex were non-significant (see Table D10 in Appendix D). Additionally, as shown in Figure 8, the three-way interaction between Sex, CS and Time was non-significant, F(1.61, 48.26) = 2.80, p = .081, ηp2 = .085, following a green-house geisser correction. As were the interactions between Sex and CS, Sex and Time, and Time and CS (see Table D11 in

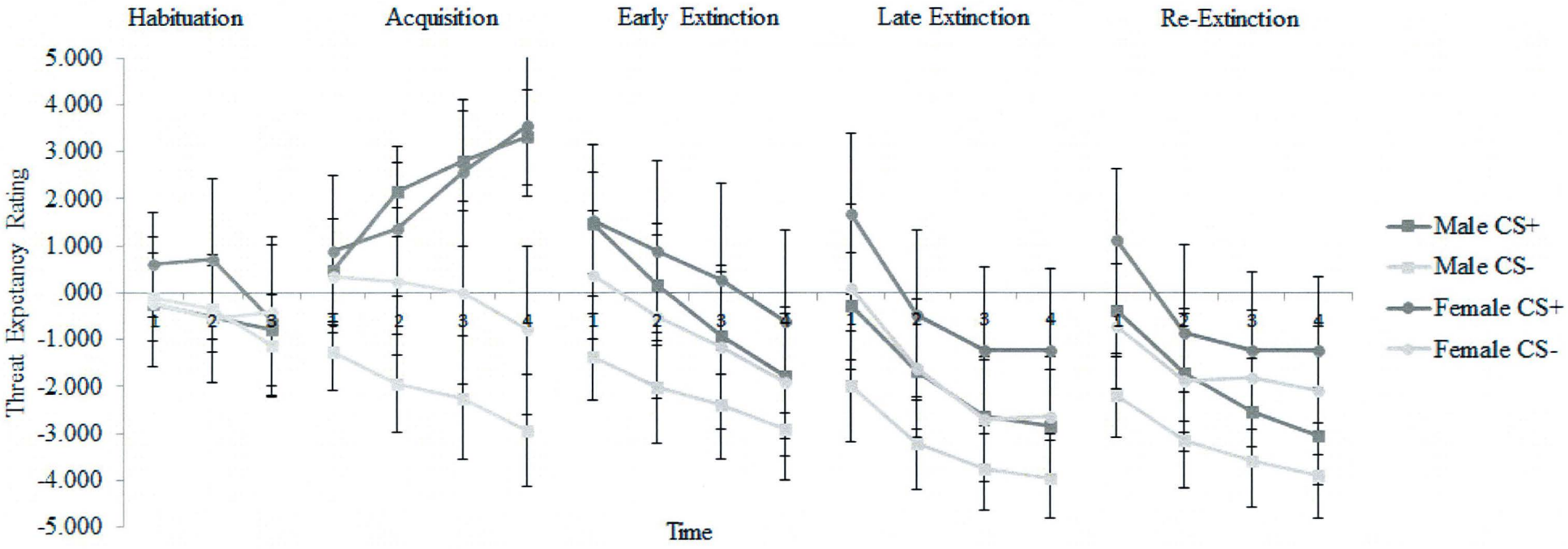


Figure 8. Threat Ratings of Each CS at each Time point for Males and Females in the Habituation, Acquisition, Early-Extinction, Late-Extinction, and Re-Extinction Phases

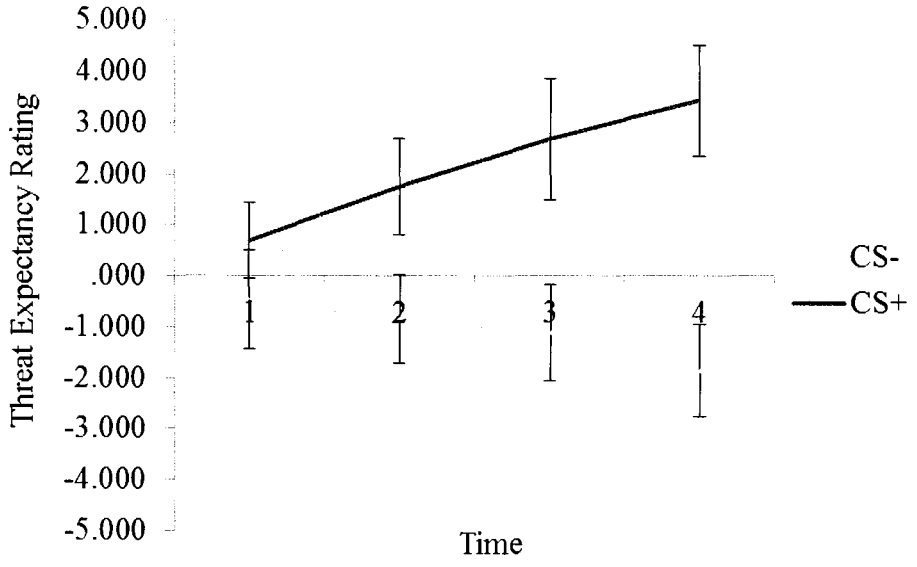
Appendix D). Therefore, these interactions were not explored further.

Acquisition. For threat expectancy, a three-way mixed factorial ANOVA revealed three significant main effects. First, a significant main effect of Sex showed that overall, the female's threat expectancy ratings ($M = 1.02$) were significantly higher than the male's ($M = 0.04$), $F(1, 30) = 4.12$, $p = .051$, $\eta_{p2} = .121$. Second, a significant main effect of CS showed that overall, participant's threat expectancy ratings were significantly higher for the CS+ ($M = 2.14$) than for the CS- ($M = -1.07$), $F(1, 30) = 25.55$, $p < .001$, $\eta_{p2} = .460$. Third, a significant main effect of Time was found, $F(1.96, 58.72) = 4.17$, $p = .021$, $\eta_{p2} = .122$, following a green-house geisser correction.

However, as shown in Figure 9, the main effects of Time and CS were subsumed by a significant interaction, $F(1.39, 41.57) = 13.41$, $p < .001$, $\eta_{p2} = .309$, following a green-house geisser correction. Sidak-adjusted pairwise comparisons broken down by Time revealed that for the CS+ threat expectancy ratings trended towards being lower at Time 1 ($M = .69$) than at Time 2 ($M = 1.76$, $p = .085$, 95% CI [-2.23, 0.09]), and were significantly lower at Time 2 than at Time 3 ($M = 2.67$, $p = .005$, 95% CI [-1.61, -0.22]) and at Time 3 than at Time 4 ($M = 3.42$, $p = .017$, 95% CI [0.10, 1.40]). Whereas, for the CS- threat expectancy ratings were not significantly different between Time 1 ($M = -.46$) and Time 2 ($M = -.84$, $p = .789$, 95% CI [-0.49, 1.25]), or between Time 2 and Time 3 ($M = -1.13$, $p = .932$, 95% CI [-0.58, 1.15]), but ratings at Time 3 were significantly higher than at Time 4 ($M = -1.87$, $p = .013$, 95% CI [-1.36, -0.12]).

As shown in Figure 8 (on page 28), the three-way interaction between Sex, CS and Time was non-significant, $F(1.39, 41.57) = 0.65$, $p = .473$, $\eta_{p2} = .021$, following a green-house geisser correction. As were the interactions between Sex and CS, and

Sex and Time (see Table D12 in Appendix D). Therefore, these interactions were not explored further.



Note: Error bars are 95% confidence intervals.

Figure 9. Threat expectancy ratings at each Time point for the CS+ and CS- in the Acquisition Phase.

Early-Extinction. For threat expectancy, a three-way mixed-factorial ANOVA showed a main effect of CS whereby, overall, participant's ratings were significantly higher for the CS+ (M = 0.14) than for the CS- (M = -1.48), $F(1, 30) = 9.43$, p = .005, η_{p2} = .239. There was also a significant main effect of Time, $F(1.43, 42.78)$ = 16.78, $p < .001$, η_{p2} = .359, following a green-house geisser correction. Sidak-adjusted pairwise comparisons revealed that, overall, threat expectancy ratings decreased over time with ratings at Time 1 (M = .52) significantly higher than at Time 2 (M = -.36, p = .021, 95% CI [0.10, 1.67]), Time 2 significantly higher than at Time 3 (M = -1.04, p = .008, 95% CI [0.14, 1.22]), and Time 3 significantly higher

than at Time 4 (M = -1.80, p = .003, 95% CI [0.21, 1.31]).

The main effect of Sex was non-significant (see Table D13 in Appendix D). Additionally, as shown in Figure 8 (on page 28), the three-way interaction between Sex, CS and Time was non-significant, $F(2.05, 61.63) = 2.50$, $p = .089$, $\eta_{p2} = .077$, following a green-house geisser correction. As were the interactions between Sex and CS, Sex and Time, and Time and CS (see Table D14 in Appendix D). Therefore, these interactions were not explored further.

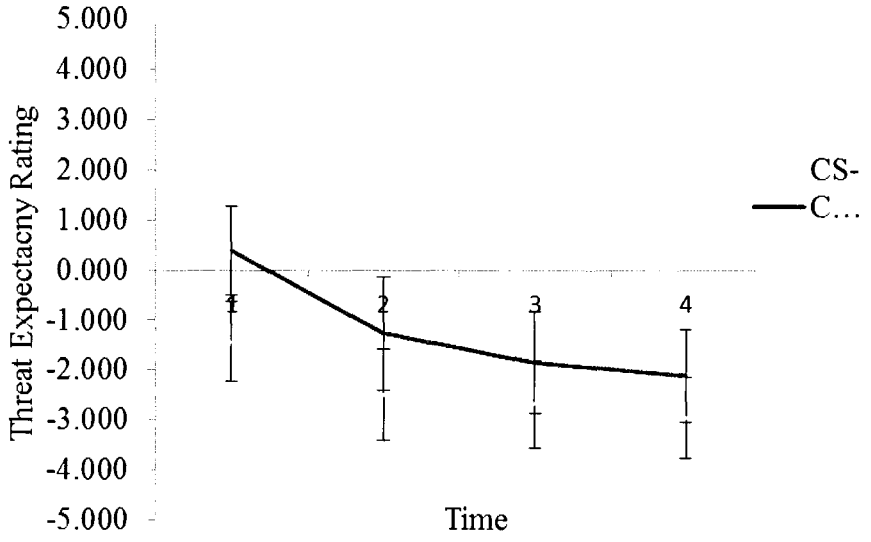
Late-Extinction. For threat expectancy, a three-way mixed factorial ANOVA revealed three significant main effects. First, a significant main effect of Sex showed that overall, the female's threat expectancy ratings (M = -1.03) were significantly higher than the male's (M = -2.55), $F(1, 30) = 4.79$, $p = .037$, $\eta_{p2} = .138$. Second, a main effect of CS showed that overall, participant's ratings were significantly higher for the CS+ (M = -1.10) than for the CS- (M = -2.47), $F(1, 30) = 8.06$, $p = .008$, $\eta_{p2} = .212$. Third, there was a significant main effect of Time, $F(1.17, 34.98) = 25.32$, $p < .001$, $\eta_{p2} = .458$, following a green-house geisser correction. Sidak-adjusted pairwise comparisons revealed that, overall, threat expectancy ratings decreased over time with ratings at Time 1 (M = -.14) significantly higher than at Time 2 (M = -1.75, $p < .001$, 95% CI [0.81, 2.41]), ratings at Time 2 significantly higher than at Time 3 (M = -2.58, p = .008, 95% CI [0.17, 1.50]), but ratings between Time 3 and Time 4 (M = -2.68, p = .830, 95% CI [-0.33, 0.14]) were not significantly different.

As shown Figure 8 (on page 28), the three-way interaction between Sex, CS and Time was non-significant, $F(1.57, 47.10) = 0.96$, $p = .373$, $\eta_{p2} = .031$, following a green-house geisser correction. As were the interactions between Sex and CS, Sex and Time, and Time and CS (see Table D15 in Appendix D). Therefore, these interactions were not explored further.

Re-Extinction. For threat expectancy, a three-way mixed factorial ANOVA revealed a trend toward a significant main effect of Sex in that the female's threat expectancy ratings (M = -1.06) were higher than the males' (M = -2.54), $F(1, 30)$ = 3.89, p = .058, η_{p2} = .115. Additionally, a significant main effect of CS showed that overall, participant's threat expectancy ratings were significantly higher for the CS+ (M = -1.21) than for the CS- (M = -2.39), $F(1, 30) = 9.94$, p = .004, η_{p2} = .249. A significant main effect of Time was also found, $F(1.64, 49.25) = 27.17$, $p < .001$, η_{p2} = .475, following a green-house geisser correction.

However, as shown in Figure 10, the main effects of Time and CS were subsumed by a significant interaction, $F(1.87, 55.96) = 4.77$, p = .014, η_{p2} = .137, following a green-house geisser correction. Sidak-adjusted pairwise comparisons broken down by Time revealed that for the CS- threat expectancy ratings were significantly higher at Time 1 (M = -1.43) than at Time 2 (M = -2.49, p = .001, 95% CI [0.35, 1.78]), but were not significantly different between Time 2 and Time 3 (M = -2.68, p = .310, 95% CI [-0.08, 0.47]), and between Time 3 and Time 4 (M = -2.96, p = .139, 95% CI [-0.60, 0.05]). Likewise, for the CS+ threat expectancy ratings at Time 1 (M = .39) were significantly higher than Time 2 (M = -1.27, $p < .001$, 95% CI [0.80, 2.52]), but ratings were not significantly different between Time 2 and Time 3 (M = -1.85, p = .153, 95% CI [-0.13, 1.29]) and Time 3 and Time 4 (M = -2.11, p = .600, 95% CI [-0.75, 0.23]).

Additionally, as shown in Figure 8 (on page 28), the three-way interaction between Sex, CS and Time was non-significant, $F(1.87, 55.96) = 0.45$, p = .626, η_{p2} = .015, following a green-house geisser correction. As were the interactions between Sex and CS and Sex and Time (see Table D16 in Appendix D). Therefore, these interactions were not explored further.



Note: Error bars are 95% confidence intervals.

Figure 10. Threat expectancy ratings at each Time point for the CS+ and CS- in the Re-Extinction Phase.

The Impact of Baseline Variables

As reported above, there were significant sex differences found on stress, anxiety, emotion regulation and baseline SCR and a trend toward significant differences on catastrophic cognitions. Therefore, multiple linear regression analyses were conducted to examine whether the sex differences found account for variance over and above that which is accounted for by anxiety, emotion regulation, baseline SCR, and catastrophic cognitions. As stress and anxiety were found to be highly correlated (r = .76), only anxiety was used in regression analyses to avoid multicollinarity. Additionally, given the recommended minimum of 10 participants for each predictor used (Green, 1991) the number of predictors selected for each regression was limited. Therefore, sex, anxiety, emotion regulation, and baseline

SCR were used as predictors for skin conductance and sex, anxiety, emotion regulation, and catastrophic cognitions were used as predictors for threat expectancy as these were considered the most relevant.

Skin Conductance. First, as reported above, for SCR in the Early-Extinction sub-phase, a significant main effect of Sex was subsumed by a significant Sex by Time interaction and sidak-adjusted pairwise comparisons revealed that males' and females' SCR differed only at Time 1 and not at Time 2, Time 3, or Time 4. Therefore, the average SCR at Time 1 was calculated and used as the dependant variable in a multiple regression analysis. As can be seen in Table 3, Sex was a significant predictor of SCR at Time 1 of the Early-Extinction sub-phase, whereas, baseline SCR, anxiety, and emotion regulation were not significant predictors.

Table 3

Predictors for SCR in Time 1 of the Early-Extinction Sub-Phase

Variables	*B*	*β*	*t*	*p*	95% Confidence Intervals	
					Lower Bound	Upper Bound
Intercept	2.30		3.60	.002	0.97	3.63
Sex	-0.58	-0.50	-3.00	.007	-0.98	-0.18
Anxiety	-0.04	-0.22	-1.08	.290	-0.10	0.03
Emotion Regulation	-0.01	-0.17	-0.85	.407	-0.02	0.01
Baseline SCR	0.01	0.02	0.11	.912	-0.14	0.16

Note: $N = 27$, $R = .74$, $R^2 = .55$, $R^2_{Adj} = .46$

Second, for SCR in the Re-Extinction phase, there was a significant Sex by CS interaction, whereby males' SCR was significantly higher than females' SCR to the CS+, but SCR to the CS- was not significantly different (see Table D17 in Appendix

D). Therefore, the average SCR for the CS+ was calculated and used as the dependent variable in a multiple regression analysis. However, as can be seen in Table 4, sex, baseline SCR, anxiety, and emotion regulation were not significant predictors of SCR to the CS+ in the Re-Extinction phase.

Table 4

Predictors for SCR to the CS+ in the Re-Extinction Phase

Variables	*B*	*β*	*t*	*p*	95% Confidence Intervals	
					Lower Bound	Upper Bound
Intercept	0.88		1.76	.091	-0.15	1.91
Sex	-0.22	-0.26	-1.30	.205	-0.57	0.13
Anxiety	0.02	0.13	0.56	.584	-0.04	0.07
Emotion Regulation	-0.01	-0.18	-0.78	.440	-0.02	0.01
Baseline SCR	0.08	0.26	1.37	.183	-0.04	0.21

Note: $N = 27$, $R = .47$, $R^2 = .22$, $R^2_{Adj} = .10$

Threat Expectancy. As is also reported above, for Threat Expectancy, there were significant main effects of Sex in the Acquisition, Late-Extinction, and Re-Extinction phases in that females' threat expectancy ratings were significantly higher than males'. Therefore, the average Threat Expectancy rating for each of these phases was used as the dependant variable in three separate multiple regression analyses. As can be seen in Table 5, Sex was a significant predictor of Threat Expectancy ratings in the Late-Extinction and Re-extinction phases but not in the Acquisition phase. However, anxiety and emotion regulation were not significant predictors in any of the phases.

Table 5

Predictors of Threat Expectancy Ratings in the Acquisition, Late-Extinction, and Re-Extinction Phases

Phase	Variables	*B*	*β*	*t*	*p*	95% Confidence Intervals	
						Lower Bound	Upper Bound
Acquisition	Intercept	-3.84		-2.90	.008	-6.56	-1.11
	Sex	0.38	0.14	0.81	.424	-0.58	1.35
	Anxiety	-0.01	-0.04	-0.17	.864	-0.18	0.15
	Emotion Regulation	0.01	0.17	0.85	.402	-0.01	0.04
	Catastrophic Cognitions	0.05	0.50	2.77	.010	0.01	0.09
	Note: $N = 27$, $R = .63$, $R^2 = .40$, $R^2_{Adj} = .31$						
Late-Extinction	Intercept	-2.20		-1.01	.320	-6.66	2.26
	Sex	2.23	0.56	2.90	.008	0.65	3.81
	Anxiety	-0.09	-0.16	-0.68	.502	-0.36	0.18
	Emotion Regulation	-0.04	-0.38	-1.74	.093	-0.09	0.01
	Catastrophic Cognitions	0.01	0.10	0.51	.616	-0.05	0.08
	Note: $N = 27$, $R = .54$, $R^2 = .29$, $R^2_{Adj} = .18$						
Re-Extinction	Intercept	-1.81		-0.76	.453	-6.69	3.07
	Sex	2.05	0.48	2.43	.022	0.32	3.78
	Anxiety	-0.04	-0.06	-0.25	.802	-0.33	0.26
	Emotion Regulation	-0.05	-0.41	-1.83	.079	-0.10	0.01
	Catastrophic Cognitions	0.02	0.12	0.57	.575	-0.05	0.08
	Note: $N = 27$, $R = .49$, $R^2 = .24$, $R^2_{Adj} = .13$						

Discussion

The aim of the current study was to examine sex differences in fear conditioning, extinction and reinstatement whilst controlling for levels of sex hormones, in particular estrogen. SCR data revealed no overall sex differences in the magnitude of fear conditioning and extinction. However, males displayed greater SCR amplitudes at Time 1 of early-extinction, which, as this was not specific to the CS+, is likely to reflect greater SCR to uncertainty. Males also displayed more rapid decrease in arousal than females in early-extinction, though again this was not specific to the CS+. Finally, males displayed greater reinstatement of fear, indexed by significantly increased SCR amplitude to the CS+. In terms of threat expectancy, females displayed greater expectancy of threat than males in the acquisition, late-extinction and reinstatement phases. Taken together, these findings suggest that males are more reactive to threat or uncertainty with SCR, have more rapidly decreasing arousal, but have a stronger arousal reaction to reinstatement, whereas, females have stronger expectancy of threat across acquisition, late extinction and reinstatement.

Validation of Fear Conditioning, Extinction and Reinstatement Paradigm

The results of this study confirmed that, as would be expected, there was no differential SCR or threat expectancy ratings to the CS+ and CS- during habituation. However, main effects of time on both SCR and threat expectancy indicated that participants habituated to the stimuli during this phase. Together, this suggests that any differences detected during later phases of the paradigm can be attributed to fear conditioning, extinction or reinstatement. Indeed, the results confirmed that differential responding to the CS+ and CS- occurred. Specifically, as would be predicted by the paradigm, participants displayed increased differential SCR and

threat expectancy ratings during acquisition, and decreased differential SCR and threat expectancy ratings during extinction, and again during re-extinction.

Acquisition. Overall, participants' SCR and ratings of threat expectancy during acquisition were significantly higher for the CS+ than for the CS- indicating that, as would be expected, participants displayed greater arousal to the CS+ than the CS-. However, the expected interaction between CS and Time was non-significant suggesting that the magnitude of the differential response to CS+ and CS- did not increase significantly over time. Specifically, participants had decreasing SCR to the CS- but a plateaued response to the CS+, indicating that SCR was particularly influenced by the safety signal (i.e., the CS-); more so than the threat signal (i.e., CS+). This suggests that a ceiling effect has occurred for SCR as reported in previous research (e.g., Bos, Beckers, & Kindt, 2012) and may be related to the 100% reinforcement schedule used in the current study. On the other hand, for threat expectancy ratings, the expected significant interaction between CS and Time was observed such that the magnitude of participants' differential threat expectancy ratings to the CS+ and CS- decreased over time. This indicates that, unlike SCR, participants threat expectancy ratings were influenced both the threat signal (i.e., the CS+) and the safety signal (i.e., the CS-). Therefore, despite the possible ceiling effect in SCR, it can be concluded that conditioning occurred.

Extinction. In the early-extinction sub-phase, participants continued to have higher SCR and ratings of threat expectancy to the CS+ than to the CS-, indicating that the greater arousal response and expectancy of threat to the CS+ continued in this sub-phase. There was also an overall decrease in SCR and threat expectancy ratings over time during this sub-phase, indicating that overall, participants' arousal and threat expectancy decreased. Taken together, this suggests that participants were

extinguishing their fear of the CS+. However, the expected interactions between CS and Time were non-significant for both SCR and threat expectancy, indicating that fear extinction did not increase significantly over time.

In the late-extinction sub-phase, there was another overall decrease in SCR and threat expectancy ratings. However, participants did not display a differential SCR to the CS+ and CS-, nor was the CS by Time interaction significant for SCR or threat expectancy ratings. This indicates that, while participants had an initial return of fear at the beginning of this sub-phase, this was likely due to general uncertainty about whether the US would be presented again and suggests that extinction had already occurred for SCR. Notably, the participants' overall threat expectancy ratings continued to be higher for the CS+ than for the CS- during this sub-phase, indicating that, unlike SCR where participants had learned to implicitly inhibit the CS/US association, participants continued to explicitly rate the CS+ as a threat signal.

Re-extinction. In the re-extinction phase, following the un-cued presentations of the US, participants' overall SCR and ratings of threat expectancy increased from the previous phase and then once again decreased over time, indicating that participants' overall arousal and threat expectancy was reinstated and then re-extinguished. Additionally, both males' and females' Also, for threat expectancy only, participants' ratings were higher for the CS+ than for the CS- and there was a significant interaction between CS type and Time such that the differential ratings of the CS+ and CS- were greater at the beginning of the phase than at the end. This indicates that participants did indeed have a reinstatement of explicit fear which they re-extinguished during this phase.

Sex Differences in Fear Conditioning

The results of this study did not provide support for the first hypothesis that

during the acquisition phase, males would be more reactive to the feared stimulus (CS+), reflected in higher levels of SCR amplitude and higher ratings of threat expectancy, compared to females. The lack of any main effect of Sex, Sex by Time or Sex by CS interactions reveals that males and females did not differ in their SCR during acquisition. However, females did rate their overall threat expectancy significantly higher than males during this phase. This suggests that there were no arousal differences in fear conditioning between males and females, but females displayed greater generalised threat expectancy, though this was not specific to the feared stimulus (CS+).

However, the Sex by Time interaction in the early-extinction sub-phase revealed that males had greater SCR amplitudes compared to females at Time 1 of early-extinction. As interpreted in previous research (e.g., Gupta et al., 2001), the initial trial of the extinction phase reflects strength of conditioning as it is prior to the extinction of the US/CS association. Therefore, this significant sex difference found at Time 1 may reflect a greater strength of conditioning in males, however, this effect was generalised and was not specific to the CS+. Therefore, a more likely interpretation is that males are generally more reactive to uncertainty (i.e., a change of phase in the paradigm) than females in terms of SCR arousal, and is consistent with the assertion by McLean and Anderson (2009) that males show greater SCR reactivity than females. Hence, together these results provide evidence that males are, in general, more reactive than females during fear conditioning, but importantly, that this greater reactivity was not specific to the feared stimuli. The results of the current study are not consistent with previous human research which found that males displayed greater SCR during acquisition, specifically to the CS+ (Guimaraes et al., 1991; Milad et al., 2006). Though, it is possible that the ceiling effect found in

SCR to the CS+ in the acquisition phase limited the sensitivity of the analysis to detect a Sex by CS interaction.

Sex Differences in Fear Extinction

The current study did not provide support for the second hypothesis that females, tested in the early-follicular phase when estrogen is low, will display impaired fear extinction, reflected in higher levels of SCR amplitude and higher ratings of threat expectancy, comparative to males. However, the findings are consistent with previous research that has failed to find within-session sex differences in fear extinction (Milad et al., 2006; Zorawski et al., 2005). In fact, most studies that have reported impaired fear extinction in females compared to males have found this effect in the recall of fear extinction, which is tested in a 2-day differential fear conditioning and extinction paradigm. This suggests that sex differences in extinction learning, and the potential impact of estrogen on extinction, are more prominent when memory consolidation processes are involved. One recent human study reported females with low estrogen had impaired within-session fear extinction (Glover et al., 2012). Although, this effect was found in a sample of women with PTSD compared to traumatized controls, and they employed fear-potentiated startle as their dependent measure, rather than SCR.

However, whilst no sex differences were found in the overall magnitude of extinction, there were some other more subtle sex differences observed. The significant Sex by Time interaction in the early-extinction sub-phase revealed that males' overall SCR decreased quickly between Time 1 and Time 2 and then plateaued, whereas, the female's overall SCR initially plateaued and then decreased between Time 3 and Time 4. This suggests that, while both sexes overall fear decreased during this sub-phase, females showed slower rates of fear reduction than

males, though importantly, this interaction did not involve a differential response to the CS+ and CS- and so cannot be taken to indicate a sex difference in extinction learning. There was also no difference between males' and females' expectancy of threat in this sub-phase.

In contrast, in the late-extinction sub-phase, there was no difference between males' and females' SCR. However, the females rated their threat expectancy higher than males, indicating that the females had greater overall threat expectancy than males in this sub-phase, indicating that the females' explicit expectancy of threat was higher than the males'.

Taken together, the sex differences found in the early- and late-extinction indicate that females displayed slower decreases in arousal, indexed by SCR, in early-phase extinction, but ongoing heightened threat expectancy in late-phase extinction, compared to males. This finding is consistent with McLean and Anderson's (2009) suggestion that females make greater assessments of threat than males and provides evidence that cognitive variables such as threat expectancy, may indeed influence extinction of fear (Sokol & Lovibond, 2012). Notably, when the males' SCR decreased so too did their cognitive threat expectancy, whereas, when the females' SCR decreased there was not an accompanying decrease in their cognitive threat expectancy.

The overall pattern found in extinction may suggest that females have a less tight coupling of their implicit arousal and their explicit threat expectancy compared to males. This is an interesting finding given that threat expectancy is often examined in the same paradigm as SCR or startle response (e.g., Bos et al., 2012; Gazendam & Kindt, 2012) and, without the inclusion of a late-extinction sub-phase, sex differences in threat expectancy would not have been found. In terms of the female

vulnerability to anxiety disorders, it is possible that female's slower fear reduction and greater expectancy of threat during extinction learning are contributing factors, though further research is required to explore this.

Sex Differences in Reinstatement of Fear

Given that no other known studies have examined sex differences in reinstatement of fear, reflected in differential SCRs and threat expectancy ratings in the re-extinction phase following the two un-cued presentations of the US, a directional hypothesis was not made. The results of the current study showed that during the re-extinction phase, there was a significant Sex by CS interaction for SCR such that males' SCR to the CS+ was significantly higher than to the CS-, whereas, females did not have a differential response. This indicates that males had a return of differential arousal response to the CS+ and CS-, suggesting that males experienced reinstatement of fear while females did not. This is likely attributable to the greater reactivity to uncertainty which was found in males' SCR compared to females' at Time 1 of the early-extinction sub-phase. In contrast, females' overall ratings of threat expectancy were significantly higher than males' following the two un-cued presentations of the US, indicating that, females had greater return of overall threat expectancy than males.

Notably, Milad et al. (2006) found sex differences in renewal, another of the four mechanisms of return of fear, and concluded that the difference was attributable to the males' greater fear conditioning. However, although renewal and reinstatement are both mechanisms of return of fear, they are not directly comparable and further research on sex differences in reinstatement of fear is required.

The Impact of Baseline Variables

Baseline sex differences were found in stress, anxiety, emotion regulation and

baseline SCR and trended toward differences in catastrophic cognitions. Therefore, to explore the possibility that the baseline differences account for the variance attributed to sex differences in fear conditioning, extinction and reinstatement, a series of multiple linear regression analyses were conducted on SCR and threat expectancy measures, with sex, anxiety, emotion regulation, catastrophic cognitions and baseline SCL included as predictor variables.

In relation to sex differences in fear conditioning, the greater SCR by males than females on Time 1 of early-extinction was further examined and it was found that sex remained a significant predictor of SCR, even once other significant group differences (i.e., anxiety, emotion regulation, and baseline SCL) were considered. On the other hand, the greater threat expectancy ratings by females compared to males during the acquisition phase was further examined and, sex was not a significant predictor of threat expectancy once other significant group differences (i.e., anxiety, emotion regulation, catastrophic cognitions) were considered. Rather, catastrophic cognitions, as measured by the CCQ, were found to be a significant predictor of threat expectancy ratings. This is not surprising given that the CCQ is also an index of generalised threat expectancy, and reinforces the importance of cognitive threat expectancy for females.

In relation to sex differences in fear extinction, the greater threat expectancy ratings by females than males in the late-extinction sub-phase were further examined. It was found that sex remained a significant predictor of threat expectancy, even once other significant group differences (i.e., anxiety, emotion regulation, and catastrophic cognitions) were considered. In relation to sex differences in reinstatement of fear, the greater SCR by males than females to the threat signal (CS+) during the re-extinction phase was further examined and it was found that sex was no longer a

significant predictor of SCR once other significant group differences (i.e., anxiety, emotion regulation, and baseline SCL) were considered. In fact, there were no significant predictors in this regression model. On the other hand, when the higher threat expectancy rating by females than males in the re-extinction phase was further examined, sex remained a significant predictor of threat expectancy once other significant group differences (i.e., anxiety, emotion regulation, and catastrophic cognitions) were considered. Therefore, on the whole, the baseline sex differences did not account for the variance attributed to sex differences in fear conditioning, extinction and reinstatement.

Theoretical and Clinical Implications

The results of the current study indicated a difference between SCR and threat expectancy. This is an interesting finding because there is an ongoing theoretical debate about whether fear conditioning and extinction are cognitively driven; in that both implicit arousal and explicit threat expectancy reflect the same underlying process (Lovibond & Shanks, 2002) or whether arousal and threat expectancy reflect separate processes underlying fear conditioning and extinction (Bechara et al., 1995; Pessiglione et al., 2008). Whilst the current study did not aim to directly test these proposed models, our findings are more consistent with the later model and with previous research that suggests implicit arousal reflects a separate underlying process than explicit threat expectancy. Additionally, and importantly, the findings of the current study suggest that this difference is particularly evident in females.

Limitations and Future Research

The Influence of Sex Hormones. The current study controlled for the influence of sex hormones by testing females during menses, when estrogen is low. Given that previous research has found effects of estrogen levels on recall of fear

extinction (e.g., Milad et al., 2006, 2010; Zeidan et al., 2011) future research would do well to further examine fear conditioning, extinction and reinstatement for females in other phases of the menstrual cycle (i.e., late-follicular phase, when estrogen is high) and females on contraceptives (e.g., Merz et al., 2011). Additionally, although testing during menses is a reliable method of ensuring low levels of estrogen, this was not confirmed using serological data and it is recommended that future studies, particularly involving other menstrual cycle phases, confirm sex hormone levels at the time of testing.

Methodological Considerations. The current study employed a 100% reinforcement schedule, meaning that every instance of the CS+ in the acquisition phase was followed by an electrical stimulus (e.g. Milad et al., 2006). However, other studies have used a partial reinforcement schedule in order to delay extinction or prevent habituation (e.g., Gazendam & Kindt, 2012). This is a possible explanation for the plateaued SCR to the CS+ observed in the acquisition phase of the current study. Therefore, it is recommended that future research uses a partial reinforcement schedule. However, notably, a similar ceiling effect was not observed for threat expectancy suggesting that threat expectancy was not impacted by habituation.

Additionally, given the variability in both SCR and threat expectancy, the sample sizes in the current study are likely to have reduced statistical power to detect effects, and future research would benefit from increased sample sizes. Also, although differential fear conditioning and extinction paradigms are routinely used in research, there is little consistency in the methodology and analysis procedures and this is likely to contribute to the lack of consistent findings in this area. This study aimed to address this by replicating the methodology used by Milad et al., (2006) and

it is recommended that future research aims to develop more consistent approaches.

Conclusion

The aim of the current study was to examine sex differences in fear conditioning, extinction and reinstatement whilst controlling for levels of sex hormones, in particular estrogen. SCR data revealed no overall sex differences in the magnitude of fear conditioning and extinction. However, males displayed greater generalised SCR following acquisition, more rapid general SCR reduction in extinction, and greater reinstatement of fear for SCR. On the other hand, females displayed greater threat expectancy ratings in the acquisition, extinction, and reinstatement phases. Taken together, these findings suggest that males are more reactive to threat or uncertainty with SCR, have more rapidly decreasing arousal, but have a stronger arousal reaction to reinstatement, whereas, females have stronger expectancy of threat. These findings are broadly consistent with previous literature (e.g., McLean & Anderson, 2009; Milad et al., 2006; Zorawski et al., 2005) and highlight an interesting differential SCR and threat expectancy response between males and females. However, further research with more consistent methodology is recommended to explore sex differences during fear conditioning, extinction and reinstatement.

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Appendices

Appendix A

Questionnaires

Appendix A1

Depression, Anxiety and Stress Scale

DASS21 *Name:* *Date:*

Please read each statement and circle a number 0, 1, 2 or 3 which indicates how much the statement applied to you *over the past week*. There are no right or wrong answers. Do not spend too much time on any statement.

The rating scale is as follows:

0 Did not apply to me at all
1 Applied to me to some degree, or some of the time
2 Applied to me to a considerable degree, or a good part of time
3 Applied to me very much, or most of the time

1	I found it hard to wind down	0	1	2	3
2	I was aware of dryness of my mouth	0	1	2	3
3	I couldn't seem to experience any positive feeling at all	0	1	2	3
4	I experienced breathing difficulty (eg, excessively rapid breathing, breathlessness in the absence of physical exertion)	0	1	2	3
5	I found it difficult to work up the initiative to do things	0	1	2	3
6	I tended to over-react to situations	0	1	2	3
7	I experienced trembling (eg, in the hands)	0	1	2	3
8	I felt that I was using a lot of nervous energy	0	1	2	3
9	I was worried about situations in which I might panic and make a fool of myself	0	1	2	3
10	I felt that I had nothing to look forward to	0	1	2	3
11	I found myself getting agitated	0	1	2	3
12	I found it difficult to relax	0	1	2	3
13	I felt down-hearted and blue	0	1	2	3
14	I was intolerant of anything that kept me from getting on with what I was doing	0	1	2	3
15	I felt I was close to panic	0	1	2	3
16	I was unable to become enthusiastic about anything	0	1	2	3
17	I felt I wasn't worth much as a person	0	1	2	3
18	I felt that I was rather touchy	0	1	2	3
19	I was aware of the action of my heart in the absence of physical exertion (eg, sense of heart rate increase, heart missing a beat)	0	1	2	3
20	I felt scared without any good reason	0	1	2	3
21	I felt that life was meaningless	0	1	2	3

Appendix A2

Catastrophic Cognitions Questionnaire - Modified

Name_____ Date_____

AGE _____SEX M_____ F_____

Instructions: The questionnaire aims at measuring your beliefs and thoughts regarding the following items. Sometimes these items are believed to be DANGEROUS. Please read the items carefully, and choose a number from the scale given below to rate the extent you believe them to be dangerous to you. Write the number you chose in the box opposite each item. For example by writing 1, you believe that the item is NOT AT ALL dangerous. By writing 5, you believe that item is EXTREMELY DANGEROUS. Do not spend too much time, and try to answer all of them.

	1	2	3	4	5	
	Not at all Dangerous	A little Dangerous	Quite Dangerous	Very Dangerous	Extremely Dangerous	
1.	Feeling edgy					
2.	Having an accident					
3.	Mind not functioning normally					
4.	Being miserable					
5.	Being injured					
6.	Unable to think rationally					
7.	Feeling shaky					
8.	Having a stroke					
9.	Unable to control thinking					
10.	Being agitated					
11.	Being ill					
12.	Losing memory					
13.	Unable to relax					
14.	Being suffocated					
15.	Being mentally blocked					
16.	Being alarmed					
17.	Being attacked					
18.	Being out of senses					
19.	Being angry					
20.	Losing sight					
21.	Being mentally blurred					

Fig. 1. Catastrophic Cognitions Questionnaire—Modified.

Appendix A3

Difficulties in Emotion Regulation Scale

Difficulties in Emotion Regulation Scale (DERS)

Response categories:

1 Almost never (0-10%)
2 Sometimes (11-35%)
3 About half the time (36-65%)
4 Most of the time (66 – 90%)
5 Almost always (91-100%)

1. I am clear about my feelings.
2. I pay attention to how I feel.
3. I experience my emotions as overwhelming and out of control.
4. I have no idea how I am feeling.
5. I have difficulty making sense out of my feelings.
6. I am attentive to my feelings.
7. I know exactly how I am feeling.
8. I care about what I am feeling.
9. I am confused about how I feel.
10. When I'm upset, I acknowledge my emotions.
11. When I'm upset, I become angry with myself for feeling that way.
12. When I'm upset, I become embarrassed for feeling that way.
13. When I'm upset, I have difficulty getting work done.
14. When I'm upset, I become out of control.
15. When I'm upset, I believe that I will remain that way for a long time.
16. When I'm upset, I believe that I'll end up feeling very depressed.
17. When I'm upset, I believe that my feelings are valid and important.
18. When I'm upset, I have difficulty focusing on other things.
19. When I'm upset, I feel out of control..
20. When I'm upset, I can still get things done.
21. When I'm upset, I feel ashamed with myself for feeling that way.
22. When I'm upset, I know that I can find a way to eventually feel better.
23. When I'm upset, I feel like I am weak.
24. When I'm upset, I feel like I can remain in control of my behaviors.
25. When I'm upset, I feel guilty for feeling that way.
26. When I'm upset, I have difficulty concentrating.
27. When I'm upset, I have difficulty controlling my behaviors.
28. When I'm upset, I believe there is nothing I can do to make myself feel better.
29. When I'm upset, I become irritated with myself for feeling that way.
30. When I'm upset, I start to feel very bad about myself.
31. When I'm upset, I believe that wallowing in it is all I can do.
32. When I'm upset, I lose control over my behaviors.
33. When I'm upset, I have difficulty thinking about anything else.
34. When I'm upset, I take time to figure out what I'm really feeling.
35. When I'm upset, it takes me a long time to feel better.

Appendix A4

Follow-up Questionnaire

Follow-up questions

• Please enter your participant code below, remember that you need to enter your initials and the last four digits of your phone number (e.g., HN2354) as you did in the screening questionnaire.

• How unpleasant was the electrical stimulus?

Not Unpleasant	Somewhat Unpleasant	Unpleasant	Quite Unpleasant	Very Unpleasant
○	○	○	○	○

• How intense was the electrical stimulus?

Not Intense	Somewhat Intense	Intense	Quite Intense	Very Intense
○	○	○	○	○

• To what degree were you frightened of the electrical stimulus?

Not Frightened	Somewhat Frightened	Moderately Frightened	Quite Frightened	Very Frightened
○	○	○	○	○

• How often was aÂ red circle followed by anÂ electrical stimulus?Â

Never	Sometimes	Always
○	○	○

•

How often wasÂ a blue circle followed by an electrical stimulus?

Never	Sometimes	Always
○	○	○

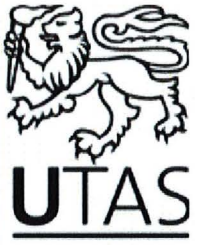
Appendix B

Ethics Approval

Appendix B1

Human Research Ethics Committee Approval Letter

Social Science Ethics Officer
Private Bag 01 Hobart
Tasmania 7001 Australia
Tel: (03) 6226 2763
Fax: (03) 6226 7148
Katherine.Shaw@utas.edu.au



HUMAN RESEARCH ETHICS COMMITTEE (TASMANIA) NETWORK

15 November 2012

Dr Kim Felmingham
School of Psychology
Private Bag 30

Student Researcher: Hollie Blackley

Sent via email

Dear Dr Felmingham

Re: FULL ETHICS APPLICATION APPROVAL
Ethics Ref: **H0012496 - Sex differences in fear extinction: The influence of cognitive variables**

We are pleased to advise that the Tasmania Social Sciences Human Research Ethics Committee approved the above project on 19 July 2012.

This approval constitutes ethical clearance by the Tasmania Social Sciences Human Research Ethics Committee. The decision and authority to commence the associated research may be dependent on factors beyond the remit of the ethics review process. For example, your research may need ethics clearance from other organisations or review by your research governance coordinator or Head of Department. It is your responsibility to find out if the approval of other bodies or authorities is required. It is recommended that the proposed research should not commence until you have satisfied these requirements.

Please note that this approval is for four years and is conditional upon receipt of an annual Progress Report. Ethics approval for this project will lapse if a Progress Report is not submitted.

The following conditions apply to this approval. Failure to abide by these conditions may result in suspension or discontinuation of approval.

1. It is the responsibility of the Chief Investigator to ensure that all investigators are aware of the terms of approval, to ensure the project is conducted as approved by the Ethics Committee, and to notify the Committee if any investigators are added to, or cease involvement with, the project.

2. Complaints: If any complaints are received or ethical issues arise during the course of the project, investigators should advise the Executive Officer of the Ethics Committee on 03 6226 7479 or human.ethics@utas.edu.au.

3. Incidents or adverse effects: Investigators should notify the Ethics Committee immediately of any serious or unexpected adverse effects on participants or unforeseen events affecting the ethical acceptability of the project.

4. Amendments to Project: Modifications to the project must not proceed until approval is obtained from the Ethics Committee. Please submit an Amendment Form (available on our website) to notify the Ethics Committee of the proposed modifications.

5. Annual Report: Continued approval for this project is dependent on the submission of a Progress Report by the anniversary date of your approval. You will be sent a courtesy reminder closer to this date. **Failure to submit a Progress Report will mean that ethics approval for this project will lapse.**

6. Final Report: A Final Report and a copy of any published material arising from the project, either in full or abstract, must be provided at the end of the project.

Yours sincerely

Katherine Shaw
Ethics Officer
Tasmania Social Sciences HREC

Appendix B2

Human Research Ethics Committee Amendment Approval Letter

Social Science Ethics Officer
Private Bag 01 Hobart
Tasmania 7001 Australia
Tel: (03) 6226 2763
Fax: (03) 6226 7148
Katherine.Shaw@utas.edu.au

HUMAN RESEARCH ETHICS COMMITTEE (TASMANIA) NETWORK

UTAS

29 April 2013

Assoc Prof Kim Felmingham
School of Psychology
Private Bag 30

Sent via email

Dear Assoc Prof Felmingham

Re: APPROVAL FOR AMENDMENT TO CURRENT PROJECT
Ethics Ref: **H0012496 - Sex differences in fear extinction: The influence of cognitive variables**

1. Addition of student investigator Mr Matthew Wade.
2. Addition of collection of saliva samples to measure estrogen, progesterone, cortisol and noradrenaline.
3. Revised information sheet, consent form and advertisement.

We are pleased to advise that the Chair of the Tasmania Social Sciences Human Research Ethics Committee approved the Amendment to the above project on 25 April 2013.

Yours sincerely

Katherine Shaw
Ethics Officer
Tasmania Social Sciences HREC

Appendix C

Information and Consent Forms

Appendix C1

Information Sheet

Participant Information Sheet

Sex differences in fear extinction: The influence of cognitive variables.

Invitation

You are invited to participate in a research study examining the influence of hormones on fear extinction. This study will be carried out in the Cognitive Neuroscience (ERP) Laboratory at the School of Psychology, University of Tasmania (Hobart). This study is being conducted by Hollie Blackley (Masters student) and Matthew Wade (Honours student), supervised by Dr Kim Felmingham in partial fulfilment of the requirements of their post-graduate studies in the School of Psychology at the University of Tasmania.

What is the purpose of this study?

The purpose of this study is to investigate the influence of hormones on fear conditioning and extinction which are key processes thought to underlie the development and treatment of anxiety disorders. Recent evidence reveals that cognitive variables and sex may influence the rates of fear conditioning and extinction, but few studies have examined the influence of hormones.

Why have I been invited to participate?

You have been invited to participate as you are a psychology first-year student and this project is being offered as part of research participation course credit. We are looking for volunteers between the ages of 18 and 45, who are not currently taking any medication, are not currently pregnant, and who have no history of psychiatric disorders. We will ask you to complete a questionnaire about these conditions before the experiment begins.

What will I be asked to do?

You will be asked to sit in a quiet room and complete some questionnaires about your mood, beliefs and cognitive processing style. You will also be asked to answer questions about your medical history, which will ask about where you are in your menstrual cycle and contraceptive use (if you are female). The study will also require taking saliva samples (collecting saliva in a small plastic tube). The samples will be examined by laboratory technicians to measure your current levels of oestrogen, progesterone, noradrenalin, and cortisol. You will then complete a behavioural task which examines how your body arousal (sweat gland activity) reacts to a mild electrical stimulus that will be administered via electrodes on the back of your hand. You will first be asked to select a level of mild electrical stimulus that feels uncomfortable but not painful to you. This will be done by attaching and delivering the lowest level of electrical stimulus, the level of which will then be increased in small increments until you report that it feels uncomfortable but not painful. You will

then be asked to complete the behavioural task. In this task, you will sit in front of a computer screen and small recording disks will be attached to your finger tips to measure your body arousal (via skin conductance). You will then be asked to watch the computer screen on which you will see different coloured circles appear. Following the presentation of some of these coloured circles, you will receive an electrical stimulus which will be set at the level you have previously chosen. You will also be asked to provide ratings on how certain you are that you will receive the electrical stimulus. The behavioural task will last approximately 25 minutes. In total, participating in this study will take approximately one hour.

What will happen to my sample after it has been tested?

Your saliva sample will only be used for the purpose of this research study. The saliva samples you provide during the study will be destroyed at the completion of the study. Your saliva samples will not be used for genetic testing or disease markers.

Will I be able to get my sample back if I want?

No, your saliva sample will be destroyed following laboratory analysis.

Will drug or biotechnology companies be able to use my sample for profit in the future?

No.

How is this study being paid for?

The study is being sponsored by a grant from the National Health and Medical Research Council.

Are there any possible benefits from participation in this study?

If you decide to participate in this research you will gain experience in research procedures and also some knowledge of underlying mechanisms of anxiety and exposure therapy. If you are enrolled in first year Psychology, you will also receive research participation credit of 1 hour for your participation. Furthermore, you will be involved in research that may provide a platform to better understand the mechanisms and processes involved in the extinction of fear, and this may ultimately lead to more efficient and effective exposure treatments for anxiety disorders.

Are there any possible risks from participation in this study?

Prior to commencement of the study you will be asked to sign consent form which will evidence your agreement to participate. You may feel a small amount of arousal or discomfort from the mild electrical stimulus. However, we expect that this arousal or discomfort to be minimal as the level that is administered will have been selected by you to be uncomfortable but not painful. The technology used to administer this electrical stimulus is very safe and has been used in many previous studies with no adverse effect reported. There will be a researcher with you at all times, and you can discontinue the study at any time without penalty and it will not affect your relationship with the University of Tasmania or the School of Psychology.

What if I change my mind during or after the study?

Participation in this research is entirely voluntary. You may choose to withdraw from

the study at any time without prejudice. Deciding to withdraw from this research at any time will not affect your academic standing in any way. You can also choose at this time to withdraw any data previously collected. You may choose to withdraw any data collected up until the 1st of July 2013. Participants will be given copies of this information sheet and the statement of informed consent.

What will happen to the information when this study is over?

Your individual data will be treated confidentially; your name will be replaced by an ID number on all data. It will be kept in a locked cabinet or on password secured computers at the School of Psychology at the University of Tasmania for a period of at least five years (with the exception of the medical questionnaires which will be destroyed on completion of the study).

Will the results of the study be published?

Following completion of the research, the data will be published. However, no participant will be personally identifiable in these publications as only group data will be published. A summary of the results of these experiments will be available on the University of Tasmania School of Psychology Web page at www.scieng.utas.edu.au/psychol or will be available by contacting the researchers from the 7th of December 2013.

What if I have questions about this study?

The researchers will be available after the testing session to answer any questions you may have. If you have any questions, or would like any additional information regarding this research please contact, Hollie Blackley at Hollie.Blackley@utas.edu.au, Matthew Wade at mwade@utas.edu.au, or Dr Kim Felmingham at Kim.Felmingham@utas.edu.au

This study has been approved by the Tasmanian Social Sciences Human Research Ethics Committee. If you have concerns or complaints about the conduct of this study, please contact the Executive Officer of the HREC (Tasmania) Network on (03) 6226 7479 or email human.ethics@utas.edu.au. The Executive Officer is the person nominated to receive complaints from research participants. Please quote ethics reference number [H12496].

Appendix C2

Consent Form

Participant Consent Form

Sex differences in fear extinction: The influence of cognitive variables.

Participant Consent Statement:

1. I agree to take part in the research study named above.
2. I have read and understood the Information Sheet for this study.
3. The nature and possible effects of the study have been explained to me.
4. Any questions that I have asked have been answered to my satisfaction.
5. I understand that the study requires me to attend the Cognitive Neuroscience laboratory at the School of Psychology where I will provide saliva samples and my arousal responses will be recorded whilst I view different coloured circles and receive a mild electrical stimulus to my hand. I understand that I can set the level of this mild electrical stimulus to feel uncomfortable but not painful prior to the task. I also understand that my involvement in this study is expected to take no longer than one hour.
6. I understand that I will be asked about recreational drug habits, use of prescription medication and my menstrual cycle and contraceptive use (if female). I also understand that I should indicate to their experimenter if I have sensitive skin and that I should request a rest if I become fatigued.
7. I understand that all research data will be treated as confidential. I agree that research data gathered for the study may be published provided that I cannot be identified as a participant.
8. I understand that my participation is voluntary and that I may withdraw from participation and/or withdraw my data at any time up until the 1st of July 2013 without prejudice to my academic standing.

Participant's name: _____

Participant's signature: _____ Date: _____

Investigator Statement
I have explained this research and the implications of participation in it to this volunteer and I believe that the consent is informed and that she understands the implications of participation

Investigator's name: _____

Investigator's signature: _____ Date: _____

Appendix D

Additional Results

Table D1

Non-significant Main Effects for SCR in the Habituation Phase

		Mean	*F*	*df*	*p*	ηp^2
Sex Main Effect	Male	.64	0.04	1, 30	.850	.001
	Female	.55				
CS Main Effect	CS-	.60	0.60	1, 30	.443	.020
	CS+	.59				

Table D2

Non-significant Interactions for SCR in the Habituation Phase

			Mean	*F*	*df*	*p*	ηp^2
Time by Sex Interaction	Male	Time 1	.72	0.25*	1.28, 38.24	.681	.008
		Time 2	.62				
		Time 3	.60				
	Female	Time 1	.61				
		Time 2	.56				
		Time 3	.47				
CS by Time Interaction	CS-	Time 1	.68	0.61*	1.40, 42.05	.953	.001
		Time 2	.59				
		Time 3	.54				
	CS+	Time 1	.66				
		Time 2	.59				
		Time 3	.52				
CS by Sex Interaction	Male	CS-	.60	2.43	1, 30	.129	.075
		CS+	.69				
	Female	CS-	.60				
		CS+	.49				

Note: * indicates green-house geisser correction.

Table D3

Non-significant Main Effect for SCR in the Acquisition Phase

		Mean	*F*	*df*	*p*	ηp^2
Sex Main Effect	Male	.66	1.33	1, 30	.258	.043
	Female	.54				

Table D4

Non-significant Interactions for SCR in the Acquisition Phase

			Mean	*F*	*df*	*p*	ηp^2
Time by Sex Interaction	Male	Time 1	.74	1.33*	1.87, 56.18	.273	.042
		Time 2	.64				
		Time 3	.68				
		Time 4	.60				
	Female	Time 1	.70				
		Time 2	.60				
		Time 3	.45				
		Time 4	.40				
CS by Sex Interaction	Male	CS-	.38	0.46	1, 30	.501	.015
		CS+	.95				
	Female	CS-	.19				
		CS+	.89				

Note: * indicates green-house geisser correction.

Table D5

Non-significant Interactions for SCR in the Early-Extinction Sub-Phase

			Mean	*F*	*df*	*p*	ηp^2
Time by CS Interaction	CS-	Time 1	.49	1.18*	1.68, 50.94	.312	.038
		Time 2	.44				
		Time 3	.38				
		Time 4	.29				
	CS+	Time 1	.78				
		Time 2	.51				
		Time 3	.53				
		Time 4	.41				
CS by Sex Interaction	Male	CS-	.54	0.16	1, 30	.901	.001
		CS+	.69				
	Female	CS-	.26				
		CS+	.42				

Note: * indicates green-house geisser correction.

Table D6

Non-significant Main Effects for SCR in the Late-Extinction Phase

		Mean	*F*	*df*	*p*	ηp^2
Sex Main Effect	Male	.47	0.69	1, 30	.414	.022
	Female	.38				
CS Main Effect	CS-	.39	0.43	1, 30	.518	.014
	CS+	.45				

Table D7

Non-significant Interactions for SCR in the Late-Extinction Phase

			Mean	*F*	*df*	*p*	ηp^2
Time by Sex Interaction	Male	Time 1	.69	0.03*	1.99, 59.67	.715	.011
		Time 2	.41				
		Time 3	.32				
		Time 4	.45				
	Female	Time 1	.66				
		Time 2	.31				
		Time 3	.26				
		Time 4	.29				
CS by Time Interaction	CS-	Time 1	.63	0.05*	1.85, 55.47	.941	.002
		Time 2	.33				
		Time 3	.26				
		Time 4	.35				
	CS+	Time 1	.72				
		Time 2	.38				
		Time 3	.32				
		Time 4	.40				
CS by Sex Interaction	Male	CS-	.41	.461	1, 30	.502	.015
		CS+	.52				
	Female	CS-	.37				
		CS+	.38				

Note: * indicates green-house geisser correction.

Table D8

Non-significant Main Effects for SCR in the Re-Extinction Phase

		Mean	*F*	*df*	*p*	ηp^2
Sex Main Effect	Male	.45	0.81	1, 30	.377	.026
	Female	.34				
CS Main Effect	CS-	.37	0.82	1, 30	.372	.027
	CS+	.43				

Table D9

Non-significant Interactions for SCR in the Re-Extinction Phase

			Mean	*F*	*df*	*p*	ηp^2
Time by Sex Interaction	Male	Time 1	.64	1.26*	1.72, 51.81	.289	.040
		Time 2	.40				
		Time 3	.34				
		Time 4	.43				
	Female	Time 1	.55				
		Time 2	.45				
		Time 3	.21				
		Time 4	.15				
CS by Time Interaction	CS-	Time 1	.62	0.67*	2.07, 62.08	.574	.022
		Time 2	.40				
		Time 3	.21				
		Time 4	.23				
	CS+	Time 1	.57				
		Time 2	.45				
		Time 3	.34				
		Time 4	.35				

Table D10

Non-significant Main Effects for Threat Expectancy in the Habituation Phase

		Mean	*F*	*df*	*p*	ηp^2
Sex Main Effect	Male	-0.51	2.25	1, 30	.145	.070
	Female	-0.08				
CS Main Effect	CS-	-0.45	0.62	1, 30	.562	.011
	CS+	-0.14				

Table D11

Non-significant Interactions for Threat Expectancy in the Habituation Phase

			Mean	*F*	*df*	*p*	ηp^2
Time by Sex Interaction	Male	Time 1	-0.17	0.03*	1.51, 48.26	.955	.001
		Time 2	-0.42				
		Time 3	-0.94				
	Female	Time 1	0.20				
		Time 2	0.08				
		Time 3	-0.50				
CS by Time Interaction	CS-	Time 1	-0.15	0.61*	1.61, 48.26	.151	.020
		Time 2	-0.45				
		Time 3	-0.76				
	CS+	Time 1	0.18				
		Time 2	0.10				
		Time 3	-0.69				
CS by Sex Interaction	Male	CS-	-0.52	2.25	1, 30	.145	.070
		CS+	-0.51				
	Female	CS-	-0.38				
		CS+	0.23				

Table D12

Non-significant Interactions for Threat Expectancy in the Acquisition Phase

			Mean	*F*	*df*	*p*	ηp^2
Time by Sex Interaction	Male	Time 1	-0.40	0.47*	1.96, 58.72	.626	.015
		Time 2	0.11				
		Time 3	0.27				
		Time 4	0.18				
	Female	Time 1	0.63				
		Time 2	0.80				
		Time 3	1.28				
		Time 4	1.38				
CS by Sex Interaction	Male	CS-	-2.10	2.84	1, 30	.102	.087
		CS+	2.18				
	Female	CS-	-0.05				
		CS+	2.09				

Table D13

Non-significant Main Effect for Threat Expectancy in the Early-Extinction Sub-Phase

		Mean	*F*	*df*	*p*	ηp^2
Sex Main Effect	Male	-1.21	2.02	1, 30	.166	.063
	Female	-0.13				

Table D14

Non-significant Interactions for Threat Expectancy in the Early-Extinction Sub-Phase

			Mean	*F*	*df*	*p*	ηp^2
Time by Sex Interaction	Male	Time 1	0.07	0.08*	1.43, 42.78	.868	.002
		Time 2	-0.92				
		Time 3	-1.65				
		Time 4	-2.34				
	Female	Time 1	0.98				
		Time 2	0.20				
		Time 3	-0.43				
		Time 4	-1.25				
CS by Time Interaction	CS-	Time 1	-0.48	1.68*	2.05, 61.63	.194	.053
		Time 2	-1.26				
		Time 3	-1.77				
		Time 4	-2.41				
	CS+	Time 1	1.52				
		Time 2	0.54				
		Time 3	-0.31				
		Time 4	-1.19				
CS by Sex Interaction	Male	CS-	-2.17	0.32	1, 30	.577	.010
		CS+	-0.25				
	Female	CS-	-0.79				
		CS+	0.54				

Note: * indicates green-house geisser correction.

Table D15

Non-significant Interactions for Threat Expectancy in the Late-Extinction Phase

			Mean	*F*	*df*	*p*	ηp^2
Time by Sex Interaction	Male	Time 1	-1.15	0.55*	1.17, 34.98	.488	.018
		Time 2	-2.44				
		Time 3	-3.19				
		Time 4	-3.41				
	Female	Time 1	0.88				
		Time 2	-1.05				
		Time 3	-1.98				
		Time 4	-1.95				
CS by Time Interaction	CS-	Time 1	-0.95	0.87*	1.57, 47.10	.402	.028
		Time 2	-2.40				
		Time 3	-3.23				
		Time 4	-3.31				
	CS+	Time 1	0.68				
		Time 2	-1.09				
		Time 3	-1.94				
		Time 4	-2.05				
CS by Sex Interaction	Male	CS-	-3.23	.000	1, 30	.995	.000
		CS+	-1.86				
	Female	CS-	-1.71				
		CS+	-0.34				

Note: * indicates green-house geisser correction.

Table D16

Non-significant Interactions for Threat Expectancy in the Re-Extinction Phase

			Mean	*F*	*df*	*p*	ηp^2
Time by Sex Interaction	Male	Time 1	-1.26	0.83*	1.64, 49.25	.421	.027
		Time 2	-2.41				
		Time 3	-3.03				
		Time 4	-3.44				
	Female	Time 1	0.23				
		Time 2	-1.35				
		Time 3	-1.50				
		Time 4	-1.63				
CS by Sex Interaction	Male	CS-	-3.18	0.08	1, 30	.777	.003
		CS+	-1.89				
	Female	CS-	-1.60				
		CS+	-0.53				

Note: * indicates green-house geisser correction.

Table D17

Sidak-adjusted Pairwise Comparisons for the Significant Sex by Time Interaction in the Early-Extinction Sub-Phase.

	Mean Male SCR	Mean Female SCR	*p*	Confidence Interval Upper Bound	Confidence Interval Lower Bound
Time 1	.89	.37	.002	0.21	0.83
Time 2	.57	.37	.197	-0.11	0.50
Time 3	.53	.38	.309	-0.14	0.44
Time 4	.48	.23	.108	-0.06	0.56

Table D18

Sidak-adjusted Pairwise Comparisons for the Significant CS by Sex Interaction in the Re-Extinction Phase.

	Mean Male SCR	Mean Female SCR	*p*	Confidence Interval Upper Bound	Confidence Interval Lower Bound
CS-	.33	.40	.608	-0.38	0.23
CS+	.58	.28	.037	0.02	0.59

Appendix E

SPSS Output

(see Disk Attached)